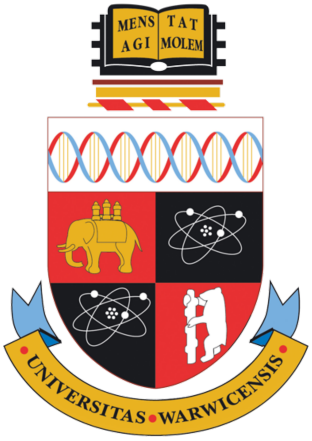

Using the Plant Trait-Based Approach to Study Temperate Grassland Ecology and Restoration

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Declaration

This thesis is submitted to the University of Warwick in support of my application for the degree of Doctor of Philosophy. It has been composed by myself and has not been submitted in any previous application for any degree.

The work presented utilised the datasets of the National Vegetation Classification, the Park Grass Experiment and the North Wyke Farm Platform. Details are presented in Chapter Two.

Summary

Grasslands are considered to be the most endangered terrestrial ecosystem in the world. In the United Kingdom, substantial losses in unimproved grasslands and the abandonment of traditional grazing has resulted in the decline of ecosystem services, such as pollination. A plant trait-based approach was conducted to study the community ecology and restoration of temperate grasslands, with a focus on the convergence/divergence patterns in response to environmental and management factors, and how these scale to the provision of ecosystem processes and services –biomass production and livestock. The role of seven plant traits, obtained from the TRY-database, was investigated using the botanical data of the National Vegetation Classification, the Park Grass Experiment and the North Wyke Farm Platform. Trait-based analyses were conducted on the latter two to investigate the provision of biomass production and livestock production. A microcosm experiment was conducted to test the effects of agricultural soil legacies and restoration seed mixture on the reassembly of grassland communities, and their associated functional structure and composition. It was found that the seven traits investigated were independent and countered the conceptualisation of ecological axes of specialisation and ecological strategies. The type of fertiliser used to improve grasslands was found to a be significant factor driving the convergence/divergence patterns of temperate grassland communities, together with grazing. Biomass production was found to be best explained by statistical models incorporating climate and environmental factors, community-weighted means and different facets of functional diversity. In essence, environmental and management pressures resembling intensely managed, especially with nitrate-based fertilisers, temperate grasslands and an exploitative community best supported greater amounts of high quality biomass. Livestock production was found to be best explained by the Functional Diversity Hypothesis: higher yields from cattle and sheep were found from diverged grazing pastures. A trade-off between cattle quantity and quality was also highlighted. Agricultural soil legacies were found to greatly hinder the progression towards vegetation and functional restoration targets, producing ruderal communities dominated by weak competitors and

opportunistic weedy plant species. The work has important implication for the management and restoration of grassland communities.

General Introduction

Humanity is believed to be existing in a new era – the Anthropocene Epoch. An era, whereby our ecological footprint far exceeds the carrying capacity of the Earth, because of historical and current anthropogenic disturbances (Kaplan, Krumhardt and Zimmermann, 2009). Many researchers have documented a wide array of anthropogenic stressors. Habitat conversion, impacting over a third of the earth's ecosystems, is recognised as the most exhaustive factor, followed by invasive species, pollution, overexploitation and climate change, all of which have degraded one third of the global ecosystems . Such actions, namely habitat loss, has produced a species' extinction rate that is one thousand times larger than the background rate, about two species per hour (Millenium Ecosystem Assessment, 2005). However, the knock-on effects this has on humanity has not gone unnoticed. In fact, the mere existence of humanity is dependent on biodiversity; "biodiversity is the life insurance for life itself" (Brownlie, King and Treweek, 2013).

Ecosystem services were defined by the Millennium Ecosystem Assessment (2005) as the benefits provided by ecosystems that contribute to making human life both possible and worth living. Biodiversity is considered the fundamental unit underpinning the delivery of goods and services. These were categorised into four distinct categories; supporting, regulating, provisioning and cultural services. It was found that globally three ecosystem services have enhanced over the last fifty years but approximately 63% are in serious decline and five hang in the balance (Millenium Ecosystem Assessment, 2005). Food production was named to have increased worldwide and was attributed to the Green Revolution in the developing work. Food was further indicated as a vital global commodity – 3% of world product.

Ecosystem services have transgressed into the socio-political sphere. There is global concerns about food security because the world population is projected to reach 9.2 billion by 2050 and food production needs to increase by 70 to 100% (Godfray *et al.*, 2010). Agricultural systems are already estimated to cover over 38% of the

world's terrestrial surfaces (Gibb, Durant and Cunningham, 2012). Historical agricultural intensification was reported to have played a key role in the conversion of more than 2/14 of the world's terrestrial biomes and more than 50% of four others. The ecosystems impacted severely include temperate, tropical and Mediterranean forests and grasslands. Recent projections suggest that a further 10-20% of these ecosystems will be converted to agriculture by 2050 (Millenium Ecosystem Assessment, 2005).

1.1. Status and Condition of Temperate Grasslands

Temperate grasslands are defined as mid-latitude grasslands that include the veldts of Africa, the pampas of South America, the steppes of Eurasia and the plain of North America. Within these systems, they can broadly be divided into three broad categories; natural, semi-natural and improved. Natural grasslands are shaped by the environment and wild herbivores, whereas semi-natural and improved grasslands have intricate relationships with ancestral and current human populations (Hejcman *et al.*, 2006). Semi-natural grasslands arose during agricultural expansion in Mesolithic to Neolithic period (Hejcman *et al.*, 2010). Improved grasslands are a by-product of contemporary agricultural improvement (Pavlů, Schellberg and Hejcman, 2011).

Globally, grasslands are said to be the most endangered terrestrial ecosystem in the world (Prober and Thiele, 2005). It is estimated that less than 10% of the world's grasslands remain, and of those approximately half are moderately or severely damaged. In the United Kingdom, a staggering 97% of grasslands have been destroyed by agricultural activities. Furthermore, 79% of semi-natural grasslands are considered to not be in a favourable condition and there are very few *in situ* surveys of semi-natural grasslands (Bullock *et al.*, 2011). Temperate grasslands in the United Kingdom are thought to exist as largely depauperate communities, which are species-poor and structurally uniform. A survey of vascular plant species found many species associated with temperate grasslands, especially calcareous and acid grasslands, had declined substantially since the 1930's (Fuller, 1987). Additionally, farmland butterfly populations were found to have declined by circa 42% (Bullock

et al., 2011). These losses to biodiversity have had major knock-on effects for the ecosystem services they supply.

Declining pollinator populations, especially *Bombus spp*, has been attributed to the intensive management of temperate grasslands. Intensive management was found to severely impact abundance and range of *Centaurea nigra* and *Trifolium pratense*, which are vital foraging plants of British bee populations (Goulson *et al.*, 2005; Carvell *et al.*, 2006). Furthermore, intensive management was found to switch temperate grasslands from a carbon sink to a carbon source. This is problematic given that British temperate grasslands are suggested to sequester carbon at rates that are higher than slow growing forest and arable land – approximately 2000kgC/ha/yr (Liebig *et al.*, 2010). This research highlights the significance of management practices to the biodiversity and ecosystem service provision from temperate grasslands.

1.2. Management of Temperate Grasslands

The management of temperate grasslands has been documented as early as 10000 BC. Large-scale deforestation in Neolithic Europe enabled human populations to establish settlements and engage in pastoralism (Kaplan, Krumhardt and Zimmermann, 2009; Hejcman *et al.*, 2013). Such has been inferred from phytolith analysis that identified ancestral communities of the grazing-tolerant *Phleum pratense*. Widespread management of temperate grasslands was conducted in the 18th or 19th centuries, which is thought to be responsible for the diversification of European grasslands. It is postulated that the combined effects of scything and livestock grazing produced pastures, meadows and grazed meadows (Pavlu *et al.*, 2007). During this era, intensification practices were prominent to boost biomass and livestock production from temperate grasslands. Hodgson *et al* (2005) indicated the intensive management of temperate grasslands resulted in an economic yield two to five times larger than unimproved grasslands.

Intensification is the process of agricultural improvement. Common practices include the addition of inorganic fertilisers and tillage followed by the sowing of

productive grass and legume species (Semelová *et al.*, 2008). European settlers in the United States and New Zealand transformed the temperate grassland communities; composition shifts from tall tussocks to short exotic grass species (e.g. *Lolium perenne*) has been widely reported throughout New Zealand and the Great Plains (Treskonova, 1991; Duncan, Webster and Jensen, 2001; Moorby *et al.*, 2006). The application of inorganic nitrogen fertilisers was found to peak in the 1980's after a number of decades; nitrogen fertilisation was suggested to have doubled since the late 1960's (Colman, Lazenby and Grierson, 1974; Lazenby, 1981). Propagation and introduction of cultivar varieties of productive grass and legume increased in the 20th century. Seed companies improved a variety of species through the artificial selection of fast germinators, leafy aboveground biomass and persistency to elevate livestock production systems (Hopkins and Wilkins, 2006; Schröder and Prasse, 2013). The intensive management strategy typically targets a single ecosystem services to optimise and in many cases that is food production. However, the intensive management strategy was found to decimate floristic richness, which had knock-on effects on invertebrate numbers and biodiversity as a whole (Pavlů *et al.*, 2012). Despite this, Bullock *et al* (2011) suggested that the agricultural improvement of temperate grasslands will play a minor role in the next thirty years. Instead, they highlighted inadequate management, such as under-grazing, as the greatest threat to temperate grasslands in the United Kingdom.

Extensification has been proposed as an alternative management strategy to intensification (Bullock *et al.*, 2011). It is premised on the cessation of agricultural improvement activities to increase the provisioning of multiple ecosystem systems, thus as multifunctional approach to temperate grassland management. This has been recommended in light of environmental initiatives, such as reducing eutrophication, boosting biodiversity conservation and carbon sequestration. Extensive management practices focus on better utilisation of agricultural by-products, such as animal manure, and boosting the overall quality of the plant community (Hopkins and Wilkins, 2006). Quality, here, can be defined according to a multitude of ecosystem service, such as the abundance of floral resources to support pollination and the grassland's aesthetic value. However, research into comparing the intensive and extensive management in relation to ecosystem processes and services is limited and needed. This is partly due to the lack of

understanding of how biodiversity translates into ecosystem functions, processes and services, and what components of biodiversity are responsible, such as genetic, species, functional and phylogenetic diversity.

1.3. The Biodiversity-Ecosystem Function Hypothesis

The Biodiversity-Ecosystem Function Hypothesis encapsulates the sampling effect, which assumes as biodiversity increases so does ecosystem functioning. A large majority of studies align with this hypothesis and employ simplistic taxon measures such as species richness; approximately 95% (Feld *et al.*, 2009). These experiments have noted a positive relationship between biodiversity and ecosystem functioning and have been praised for eliciting the association between environmental change and biodiversity (Balvanera *et al.*, 2006). The focus on species richness was suggested to represent real-world biodiversity, but many authors urge the movement away species richness to better solve the relative contributions of the various components on ecosystem processes and services (Mouillot *et al.*, 2011). Harrison *et al* (2014) concluded that the Biodiversity-Ecosystem Function relationship is complex with a high degree of uncertainty. The building of a predictive framework has also been urged. An extremely influential biodiversity component was highlighted in the 1990s – functional diversity (Tilman *et al.*, 1997). This was suggested to have revolutionised the Biodiversity-Ecosystem Hypothesis and the opened new line of investigations ecological subdiscipline – Functional Ecology. Functional based approaches have now gained considerable momentum in the fields of agronomy, forestry, conservation, archaeobotany and evolution (da Silveira Pontes *et al.*, 2015; Martin and Isaac, 2015).

1.4 Functional Ecology

The popularisation of functional ecology has attracted much theoretical and practical interest. Trait-based approaches have been hailed as the 'Holy Grail' by Lavorel & Garnier (2002) as they believed a trait-based approach paves the way to a unified theory of community ecology and ecosystem service science, which is capable of revealing and predicting community processes from functional traits. Consequently, this facilitates the assessment of interactions between species, their

environments, their distribution, assembly and effects on ecosystem functioning (Garnier and Navas, 2012). This thesis focusses solely on plant functional traits but higher trophic trait-based approaches do exist – notably with regards to animal guilds. Pérez-Harguindeguy *et al* (2013) defined plant functional traits "to be any morphological, physiological, or phenological feature, measurable for individual plants, at the cell to the whole-organism level, which potentially affects its fitness". Plant functional traits are, therefore, related to components of growth, reproduction, and survival (Craine *et al.*, 2001).

Currently, the largest plant trait database (TRY) records approximately 1800 plant trait values and recent developments have seen the introduction and publication of a thesaurus of plant characteristics for ecology and evolution documenting the name, definition, formal units and synonyms for more than 700 plant traits (Kattge *et al.*, 2011; Garnier *et al.*, 2017). Early examples of plant traits categorised plant species according to life and growth forms, such as Raunkiær plant life-form, but now are also measured on continuous and ordinal scales. Furthermore, Weiher *et al* (1999) introduced the hard/soft terminology to define plant traits. Hard was defined as traits that are ideal and would adequately represent the function of interest, but are difficult to measure, and therefore a surrogate trait (an easy trait) is employed (Violle *et al.*, 2007a). For example, understanding a species' dispersal strategy requires definition of the spatial (dispersal distance) and temporal (propagule longevity) parameters or hard traits, however such are impractical and so easy traits on continuous (seed mass) or categorical (dispersal mode and seed shape) can be implemented to adequately represent a species' dispersal strategy (Weiher *et al.*, 1999).

Recent decades has seen a general movement away from categorical and ordinal plant traits and the implementation of continuous traits in practice (Maire *et al.*, 2015). This can be attributed to the widespread compilation of plant traits into databases that increase accessibility and reduces labour in the field (Kattge *et al.*, 2011). Research, therefore, has focused on calculating mean trait values for the species of interest without *in situ* measurements (Cornwell and Ackerly, 2009).

Questions have arisen, however, regarding the robustness of this assumption with respect to intraspecific differences (Valladares, Gianoli and Gómez, 2007).

1.4.1. Intraspecific Trait Variation

Intraspecific trait variation is believed to reflect an individual's *ad hoc* adaptations to local environmental pressures (Valladares, Gianoli and Gómez, 2007). For example, Siefert *et al* (2014) reported that intraspecific variation in vegetative height conferred fitness increases when soil phosphorus was plentiful. Researchers agree that accounting for intraspecific trait variation is not economically viable in circumstances where there are numerous plant traits, species and ecosystems under investigation. Albert *et al* (2011) suggested determining intraspecific trait variation at fine organizational or spatial scales because it is likely to be more important than interspecific differences. This assumption, however, is also dependent on the plant traits in question. Seed mass is suggested to be phylogenetically conserved within a species, whereas foliar traits are postulated to be plastic (Garnier and Navas, 2012; May, Warner and Wingler, 2017). Intraspecific variation in specific leaf area was found to range from 13 to 30% and leaf dry matter content ranged from 8 to 20% especially across geographical temperature gradients (Harzé, Mahy and Monty, 2016; May, Warner and Wingler, 2017). On the other hand, Messier, McGill and Lechnowicz (2010), however, interspecific and intraspecific to be equal at the leaf, tree, strata and site level for leaf dry matter content.

The literature presented embodies the inconsistencies in the current understanding in functional ecology with regards to intraspecific trait variation. When and where to account for intraspecific trait variation is still highly contested, but Albert *et al* (2010) published some guidelines that can help direct investigations. In essence, spatial scale is the key determinant and small-scale investigations should concentrate on measuring plant trait values at the individual level. Albert *et al* (2010) does advocate for the use of mean trait values at macro and global scales. There is great utility in trait databases in examining global relationships between traits and the formulation of ecological axes of specialization and their associated strategies.

1.5. Ecological Axes of Specialization

Ecological axes of specialisation are defined as dimensions that reflect how plant functional traits co-vary (Schellberg and Pontes, 2012). These trait syndromes are believed to be the phenotypic expressions of abiotic and biotic evolutionary processes that have shaped the fundamental niches of species (McGill *et al.*, 2006; Violle *et al.*, 2015). This has perpetuated the notion that ecological axes of specialisation are conserved in particular plant lineages and classifying plant species according to plant life form or growth form can adequately define a plant species fundamental niche (Lavorel *et al.*, 1997). However, such classifications have been heavily criticised for ignoring the importance intra-lineage variation, and the inability to define ecological strategies based on purely phylogenetic data (Mitchell and Bakker, 2016). Grime's C-S-R triangular theory was a pioneering, and still heavily cited, ecological strategy that tackled elements of species' growth, reproduction, and survival.

1.5.1. C-S-R Triangular Theory

This theory hypothesises that that there three major classes of vegetation (C, S and R) elicit different strategies to stress and disturbance. Grime (1979) defined stress as "the external constraints which limit the rate of dry matter production of all or part of the vegetation", such as shortages of light, water and mineral nutrients, or sub-optimal temperatures (Grime, Hodgson and Hunt, 2007). Disturbance was indicated "the mechanisms which limits plant biomass by causing its partial or total destruction" – herbivory, pathogens, mowing, drought, erosion and fire. It was assumed that it was impossible for any species to grow in the combination of high stress and high disturbance. Grime defined the three classes of vegetation according to field observations and studies of plant life-history traits, theories of plant competition for resources and screening of plant traits (Grime, Hodgson and Hunt, 2007). C-selected 'competitors' are able to thrive in relatively stable (low stress, low disturbance), productive habitats via investment of resources in continued vegetative growth and rapid attainment of large individual and organ size to aid resource pre-emption. S-selected 'stress tolerators' exits in resource-poor environments, and thus invests more metabolic energy into retaining resources and repairing cellular components in dense, long-lived tissues. R-selected 'ruderals' have

a higher degree of reproductive investment to ensure population regeneration in response to continual and potentially lethal disturbances events. The theory of functional equilibrium is central to the CSR and assumes vegetation can only develop in spatial and temporal spaces that are a result of equal intensities of stress, disturbance and competition. (Grime, Hodgson and Hunt, 2007). This optimal positioning represents a species unique place in the community – its niche (Kattenborn *et al.*, 2017).

The C-S-R triangle theory has informed a great deal of plant functional investigations. Early research relied on nominal categories, such as classes of flower period, lateral spread and canopy height to assign a species' to a vegetation class (Grime, Hodgson and Hunt, 2007). Westoby (1998), however developed the Leaf-Height-Seed Scheme on the foundations of Grime's theory using continuous plant traits. Using plant height, specific leaf area and seed mass. Westoby (1998) demonstrated the application of ecological axes to functional trait ecology. The relationship of these traits to the C-S-R theory are illustrated in Figure-1.5.1. The Leaf-Height-Seed Scheme supplemented Grime's CSR and questioned the viability of individuals at high S and R strategies, whilst assuming the independence of its core three traits (the greyed-out region on Figure-1.5.1.). Since, the Leaf-Height-Seed Schemes has been divided into three spectra (Leaf Economics, Plant Height and Seed Size) (Peter J. Wilson, Thompson and Hodgson, 1999; Garnier and Navas, 2012). The Leaf Economics Spectrum is notably the most widely accepted and has been noted across different spatial and ecological scales, such as aquatic plant species inhabiting the subarctic ecosystem (Freschet *et al.*, 2010). Pierce *et al* (2013) revised Grime's CSR using continuous plant traits and suggested grounding the CSR in the leaf economics spectrum. Furthermore, two global studies investigating the patterning of plant traits and their co-variation as ecological strategies confirmed the prevalence of the Leaf Economics Spectrum (Díaz *et al.*, 2004, 2015). The findings indicated that the Leaf Economics Spectrum is phylogenetically and geographically conserved, and such has been reported in certain biomes (Pierce *et al.*, 2017). The number and identity of plant traits aligning to the Leaf Economics Spectrum is continually expanding.

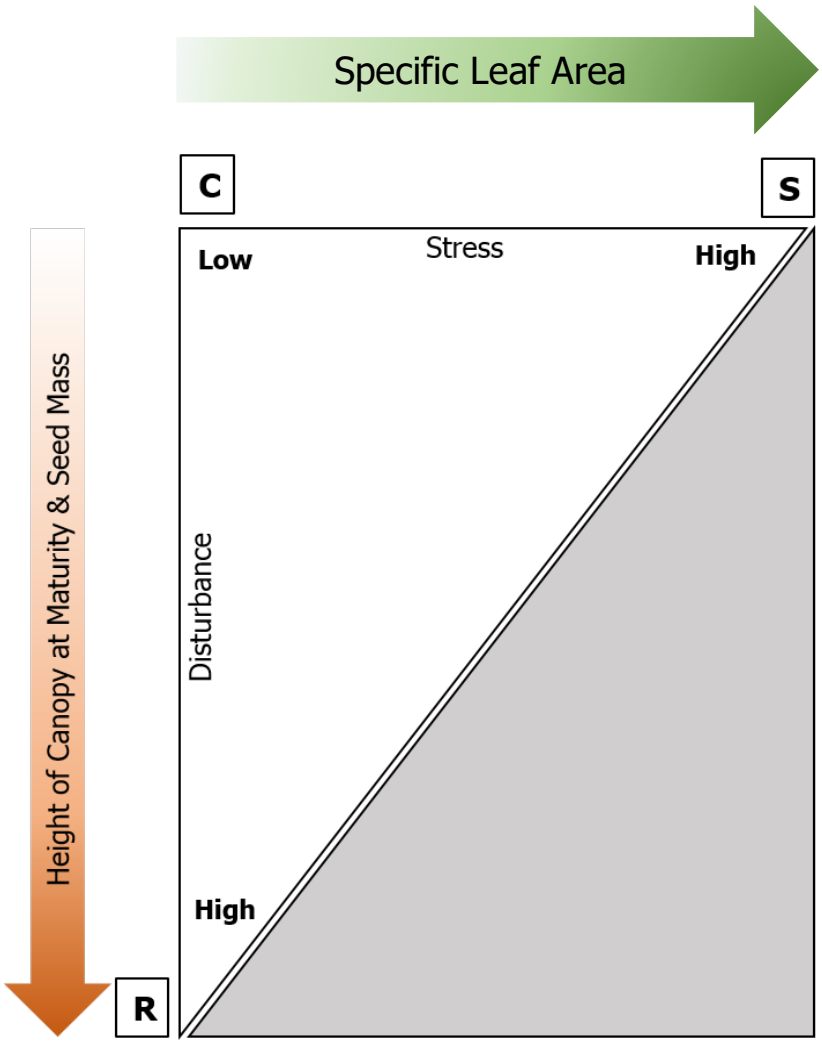

Figure-1.5.1: Annotated Grime's C-S-R triangular theory with the dimensions of the Leaf-Height-Seed.

Westoby (1998) first identified leaf mass area and its inverse specific leaf area to be the core plant traits of this spectrum and was associated with the establishment and persistence of plant communities. Typically, specific leaf area is viewed as synonymous with a species' relative growth rate and is suggested to impact a species' establishment via seedling relative growth rate and persistence through the ability to quickly respond to disturbances. Westoby *et al* (2002) conceptualised these notions into an ecological strategy categorising species and individuals as exploitative or conservative. Given the far-reaching nature of the Leaf Economics Spectrum, researchers have identified a whole host of plant traits that display allometric and trade-off relationships with specific leaf area. Craine *et al* (2002) noted that these span morphological, metabolic and biochemical plant traits.

Specific leaf area was found to be associated with leaf physical strength, leaf thickness, leaf dry matter content and leaf nitrogen content. Increased specific leaf area invests fewer resources in the construction of physical structures, reducing leaf physical strength, thickness and dry matter content (Westoby *et al.*, 2002). The thinner leaves, in turn, facilitate carbon dioxide diffusion to chloroplasts rich in RuBisco and metabolic components conducive to photosynthesis rate (Weiher *et al.*, 1999; Wright *et al.*, 2005). Resources are thus suggested to be invested in capturing further resources and is characteristic of the exploitative strategy. Conservatives, on the other hand, produce leaves adapted to endure long periods with reduced metabolic activity (Lavorel and Grigulis, 2012).

The Leaf Economics Spectrum has undoubtedly enriched the understand of community ecology and provided working models of how ecological strategies are dictated by environmental conditions, and how these translate into ecosystem processes and services (Violle *et al.*, 2007a). Maire *et al* (2009), for example, measured the trait profiles (leaf nitrogen content, root uptake capacity and leaf nitrogen use efficiency) and classified thirteen perennial grass species of montane grasslands according to responses along a soil nitrogen gradient. Their resulting typology mirrored that of Westoby (1998); nitrogen-exploitative and nitrogen-conservative strategies. The exploitative strategy has also been found to result in increased community productivity and higher yields of biomass (Garnier *et al.*, 2004; Duru *et al.*, 2010). Understanding the complex relationships between plant traits, the environment and the production of ecosystem processes and services was simplified by the Response-Effect Framework proposed by Lavorel and Garnier (2002).

1.6. The Response-Effect Framework

Lavorel and Garnier (2002) developed a conceptual and methods to understand the associations between abiotic and biotic factors and species traits and to predict changes in ecosystem processes and services. The framework amalgamated the deterministic view of community assembly with niche concepts and assumed the structure and composition of plant communities is assembled non-randomly according to hierarchy of filters that select species from the regional pool according

to their response trait(s) (Keddy, 1992; Götzenberger *et al.*, 2012). The succeeding plant community then displays an effect trait profile that modulates ecosystem processes and services. These concepts of response and effect traits are illustrated in Figure-1.6.1 and explored in 1.6.1. and 1.6.2. respectively.

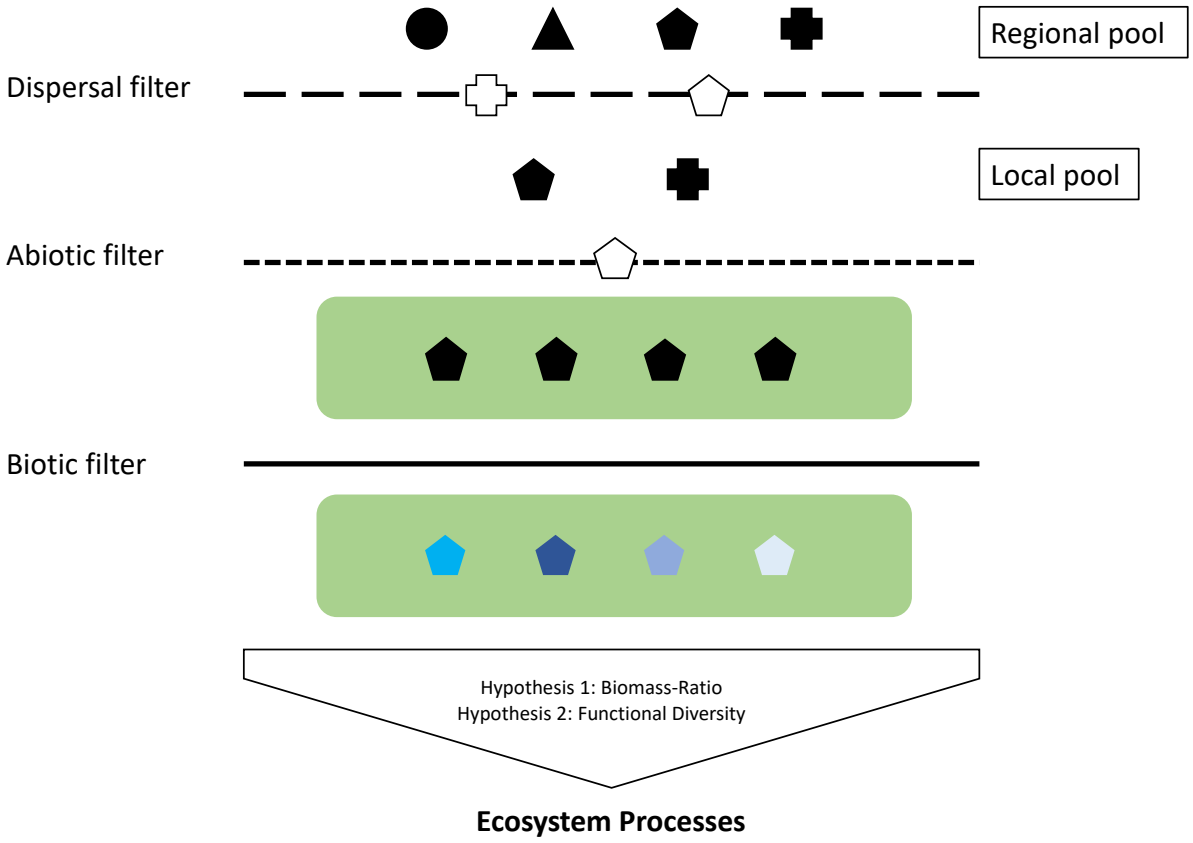

Figure-1.6.1: Conceptual model of the Response-Effect Framework. Black shapes indicate individual plant trait values. White shapes illustrate the positively selected plant trait value at each filter. The different hues of blue demonstrate the action of the biotic filter whereby trait values differentiate in order for species to coexist.

1.6.1. Response Traits

Response traits refer to plant features that show consistent responses to particular environmental factors and therefore reflect adaptations to abiotic and biotic conditions. They are theorised to be subjected to three filters, operating at different spatial scales (Figure-1.6.1.). The dispersal filter limits the potential regional pool of colonists based on geographical barriers. This regional pool is further refined by an

abiotic filter, which is an expression of the environmental and disturbance conditions acting at the local level, and finally the biotic filter that impacts the establishment of individuals, negatively through competition or positively through facilitation. The abiotic filter converges the values of plant functional traits, which is suggested to represent the performance optimum in the community (local adaptation) (McGill *et al.*, 2006). Performance optima have been experimentally investigated through environmental gradient analysis, whereby transformed species abundance data into community-weighted plant trait values are analysed along environmental gradients (Laughlin, 2014b). Peaks in these optima along environmental gradients is suggested to reflect a species' fundamental or functional niche – a species' unique position along an environmental gradient within a single or multidimensional trait space (Violle and Jiang, 2009).

Species co-existence is achieved when species have differential performance optima suggesting distinct fundamental niche preferences (McGill *et al.*, 2006). Key abiotic filters in grassland communities include site legacies of previous land-use, as well as altitude, soil fertility, topographically mediated gradient in water availability and temperature (Baeten *et al.*, 2010; Lavorel and Grigulis, 2012). Cornwell & Ackerly (2009) found that water availability strongly impacted within-community distribution of leaf, stem and belowground traits of plant species; specific leaf area, vegetative height, and wood density. Soil fertility gradients are the most common examined in grassland ecology and the effects on plant functional trait values are well-defined. These are traditionally honed in on the leaf economics spectrum and the identification of exploitative and conservative strategies to nutrient acquisition and use (Wright *et al.*, 2004). Nutrient-rich grasslands lead to convergence towards higher specific leaf, tissue nitrogen concentration and reduced leaf dry matter content and closely resembles the profile of the exploitative strategy (Lavorel and Grigulis, 2012). Response trait studies have predominantly concentrated on the soil nitrogen gradients under the assumption that all soil nutrients co-vary along this gradient.

The abiotic filter hypothetically produces a local species pool that share the same fundamental niche, and therefore, species interactions ensue at the local level

(McGill *et al.*, 2006). This biotic filter is regarded as a divergence force. The 'limiting similarity' hypothesis assumes dominant competitors largely occupy the niche space, and co-existence is only possible upon differentiation in resource acquisition and use strategies. Da Silveira Pontes *et al* (2015) reported differential root uptake capacities in grass species in response to soil nitrate and ammonium. Dominant species were found to prefer nitrate and rarer species were suggested to have differentiated to exploit the less favoured ammonium. To understand a species' competition potential, research has begun to construct competitive hierarchies and calculate competition coefficient based on functional traits (Keddy, Twolan-Strutt and Shipley, 1997; Laughlin, 2014a). Plant height has been central to these investigations and is inferred to play a significant role in the competition and interception of solar radiation (Park, Benjamin and Watkinson, 2003).

1.6.1a. Modelling Approaches to Abiotic Filters

Mathematical niche trait-based models are widely accepted in to assess abiotic filtering. The MaxEnt (maximum entropy) model was first developed by Shipley, Vile and Garnier (2006) and is thought to have inspired a resurgence in modelling species abundances (Laughlin *et al.*, 2012). In essence, the MaxEnt model assumes non-random assembly of species through a series of abiotic filters that shape the functional structure of a community. Relative species abundances are predicted according the species pool, species' mean trait values and recorded community level mean trait values (Shipley, 2010). The relative abundance of a species is therefore a function of how closely its mean functional trait values agree with the community level mean (Shipley, Vile and Garnier, 2006). There is considerable empirical support for the MaxEnt model and has been successful in dictating non-random assembly processes for a whole suite of plant traits (Sonnier, Shipley and Navas, 2010). The model, however, has been heavily scrutinized for ignoring intraspecific trait variation (Albert *et al.*, 2010; Laughlin *et al.*, 2012). Laughin *et al* (2012) proposed a hierarchical Bayesian model (Traitspace), which combined intraspecific trait variation, ecological axes of specialization and abiotic filters. The Traitspace model has provided novel opportunities to quantify the strength of specific abiotic filters, the joint effects of multiple pressures and unveil the actions of the biotic filter (Laughlin *et al.*, 2012, 2015). The latter is a great development

for functional ecology as researchers continually struggle to study the biotic filter in isolation and determine the resulting plant trait structure and composition.

1.6.2. Effect Traits

The Response-Effect Framework attempts to mechanistically associate environmental factors and plant traits with ecosystem functions, processes and services (Lavorel and Garnier, 2002). Early accounts centred on defining the ecosystem processes and services characteristic of the exploitative and conservative strategies underpinned by the Leaf Economics Spectrum (Wright *et al.*, 2004). In essence, exploitative plant species were found to increase nutrient turnover and litter decomposition rates and conservatives were thought to support carbon sequestration and soil water retention (Gross, Suding and Lavorel, 2007; Lavorel and Grigulis, 2012; Grigulis *et al.*, 2013). The use of ecological strategies, however, has demonstrated inconsistencies but they have been incredibly useful in pinpointing the identity of plant traits likely to impact a whole host of ecosystem processes and services. Instead, the scaling of functional structure and composition to ecosystem processes and services has translated into two hypotheses; the Biomass-Ratio Hypothesis and the Functional Diversity Hypothesis (Craine *et al.*, 2001; Lavorel and Garnier, 2002).

The Biomass-Ratio Hypothesis predicts the delivery of ecosystem goods and services is determined by the trait profiles of the most abundant species (Grime, 1998). Community-weighted means (the average trait value per unit biomass) exemplify this hypothesis and has been implemented extensively in plant effect traits and ecosystem processes and services research. Community-weighted means of plant height and leaf dry matter content have been shown to drive primary productivity and decomposition of litter respectively (Lavorel and Grigulis, 2012). The latter, in fact, was found to underpin herbivore productivity from temperate grassland systems (Pakeman, 2014b). In general, the Biomass-Ratio Hypothesis is regarded to be stronger determinant than Functional Diversity Hypothesis (Laliberté and Tylianakis, 2012).

The Functional Diversity Hypothesis is grounded by the original conceptions of the Biodiversity-Ecosystem Function Hypothesis. The sampling effect plays a pivotal such that higher functional diversity represents a greater range of trait values, and filled niche spaces, which guarantees the maintenance of ecosystem processes and services through functional redundancy (Yachi and Loreau, 1999; Firn, 2007; Mokany, Ash and Roxburgh, 2008). Functional diversity has been deconstructed into three complementary concepts; richness, evenness and divergence, which describes the distribution of species in an n-dimensional niche according to its size and occupancy (richness), abundance patterning (evenness) and distribution relative to the community centroid (divergence) (Villéger, Mason and Mouillot, 2008; Mason *et al.*, 2013). The provisioning of ecosystem services is, therefore, consistent and predictable when a n-dimensional niche space is broad and evenly exploited. The functional diversity hypothesis has found support from a wide range of ecosystem processes and services, such as wood production, carbon sequestration, soil nutrient retention and pollination, but research is challenged on calculating functional diversity from a univariate or multivariate trait space (Díaz, S. Lavorel, *et al.*, 2007).

Villéger, Mason and Mouillot (2008) proposed three multivariate functional diversity indices mathematically summarising the concepts of richness (FRic), evenness (FEve) and divergence (FDiv). Since, this repertoire has expanded considerably particularly with regards to functional divergence; Rao's Q and functional dispersion (Botta-Dukát, 2005; Laliberté and Legendre, 2010). Furthermore, univariate functional indices have been devised to quantify the functional evenness and divergence of single traits (Mouillot *et al.*, 2005). Approximately forty indices are circulating with the literature and functional ecologists are tasked with subjectively choosing the most robust and complementary indices in practice (Mouchet *et al.*, 2010). Single trait analyses have indicated their utility in predicting biomass production from temperate grasslands, however, the effects are highly contested and in some cases contradictory of the sampling effect that underpins the functional diversity hypothesis. Grigulis *et al* (2013) found a negative effect of divergence in canopy on biomass production. For multivariate indices, research is still relatively sparse but continued implementation and study has been recommended (Clark *et al.*, 2012).

1.7. Thesis Aims and Hypotheses

Plant functional trait ecology is still relatively embryonic, and challenges are still ahead. Further research into every aspect of functional ecology has been urged particularly ecological axes of specialisation and the Response-Effect Framework (Lavorel and Garnier, 2002). With regards to grassland management and restoration, functional relationships between available resources, plant functional traits and ecosystem services are rarely considered in practice (da Silveira Pontes *et al.*, 2015). With these directions in mind, this thesis aims investigate whether a trait-based approach can determine the key factors of community composition and ecosystem services provision of temperate grasslands. This is achieved by satisfying the aims and hypotheses of the four experimental chapters, presented below.

1.7.1. Chapter Three:

- The aim was to investigate the underlying ecological axes of specialisation that are specific to temperate grasslands. Two hypotheses were examined:
    - Hypothesis 1: Evidence for the Leaf Economics Spectrum, the Plant Height Axis and Seed Mass Axis will be found in temperate grassland communities.
    - Hypothesis 2: Functional richness, evenness and divergence will be independent.

1.7.2. Chapter Four

- The aim was to provide new information on the convergence-divergence paradox in relation to environmental and management factors of temperate grasslands. Three hypotheses were investigated:
    - Hypothesis 1: The plant traits significantly impacted by grazing intensity will display a profile aligned to the grazing tolerance strategy; increasing grazing intensity will cause convergence.
    - Hypothesis 2: Improved temperate grasslands will exhibit a trait profile and convergence-divergence pattern typical of the exploitative strategy, irrespective of organic or inorganic improvement.
    - Hypothesis 3: Mineral fertilisation will have similar influences than nitrogen-based fertilisation on plant trait structure and composition.

1.7.3. Chapter Five

- The aim was to examine the mechanisms that support the provision of ecosystem services from temperate grasslands. Four hypotheses were investigated:
    - Hypothesis 1: The combination of inorganic nitrogen fertilisation and dominant species possessing traits related to the Size Axis (plant height and seed mass) will explain the most variation in the quantity of forage material.
    - Hypothesis 2: Dominant species with palatable and digestible traits (high leaf nitrogen content, low leaf dry matter content and leaf C:N) will underpin the quality of forage material, and climate and environmental factors will play an auxiliary role.
    - Hypothesis 3: Climate and environmental variables and dominant species with palatable and digestible traits, predominantly leaf dry matter content, will be significantly and positively related to the quantity of livestock outputs.
    - Hypothesis 4: Compounds associated with nutritional value, such as leaf nitrogen content, will be a key variable in predicting the quality of livestock products.

1.7.4. Chapter Six

- The aim was to investigate the trait-based assembly of temperate grassland communities and understand the impacts of abiotic and biotic factors during seed germination and establishment, and their effects on success in the early stages of temperate grassland restoration experiment. Three hypotheses were examined:
    - Hypothesis 1: Seed and germination traits will feature in the optimal Maximum Entropy Model together with mature plant traits.
    - Hypothesis 2: The seed and germination trait profile of dominant species will reflect a fast germination strategy in response to a strong agricultural legacy.
    - Hypothesis 3: Soils with a strong agricultural legacy will greatly hinder the progression towards both vegetation and functional targets.

Overview of the Experimental Platforms and Plant Trait Databases

2.1. Introduction

The overarching aim of this thesis, to examine whether a trait-based approach can reveal the determining factors of community composition and ecosystem services in temperate grasslands, was achieved using data collected from two experimental platforms – the Park Grass Experiment and North Wyke Farm Platform – controlled by the academics of Rothamsted Research. The data gleaned were used to construct three matrices: site x species abundances, site x environmental variables, and site x ecosystem service measures. Plant functional trait data was requested from the TRY plant trait database. In this chapter, an overview of the experimental platforms and the data mined from them is provided, together with explanations concerning data processing and preliminary analyses of spatial autocorrelation. The TRY plant database is described and methods of dealing with missing trait values are reviewed. Finally, an illustrative summary of the methodology is provided to give a complete insight of the datasets and statistical analyses of Chapters 3, 4 and 5.

2.2. The Park Grass Experiment

The Park Grass Experiment is the world's oldest ecological experiment. It was established in 1856 by Sir John Lawes at Rothamsted, Hertfordshire, UK on approximately 208 hectares of parkland thought to have been a species-rich pasture for some centuries. The initial aims were to investigate approaches to improving yield from a hay-meadow grassland by the application of different fertiliser regimes (both inorganic and organic) (Silvertown *et al.*, 2006). Ever since, the Park Grass Experiment has attracted scholars and inspired scientific literature that has enriched ecological study and provided insights for nature conservation.

The area is described as naturally well-drained with a heavy loam topsoil, a clay subsoil that is rested upon chalk and it was initially divided into seventeen plots

with differing fertiliser regimes. Over the last 160 years, the treatment structure of the Park Grass Experiment has evolved; there are now sub-plots with a unique fertiliser and liming schedule to achieve target pH values of five, six and seven together with an unlimed plot. Currently, the Park Grass Experiment resides in its third phase (since 1965); twenty plots, each with an area between 0.05-0.2 hectares and a plethora of different fertiliser regimes (Figure-2.2.1 and 2.2.2.).

The different fertiliser and liming treatments have transformed the once uniform species-rich hay-meadow with regards to botanical structure and composition. Park Grass Experiment is a mosaic of plots that differ in species richness, composition and annual net primary production, yet they share the same microclimate and original soil type (Silvertown *et al.*, 1994). These plots, however, are now believed to be in a state of dynamic equilibrium (Silvertown *et al.*, 2002). Silvertown (1980) suggested that community equilibrium was reached forty years after establishment, as the botanical composition of three floristic guilds (grass, legumes and miscellaneous species) was stable in annual hay cuts. The dynamism manifests itself at the species-level, as species composition within these guilds has continued to change over time and with fertiliser and liming treatments. Despite reaching dynamic equilibrium, species outbreaks have been reported within communities of stable biomass and consistent guild compositions. These outbreaks were suggested to be species responses to temporary increases in resource availability (Silvertown *et al.*, 2002). Habitat characteristics were found to be better discriminators of outbreak species and fecundity traits, such as mating system (out-crossing vs inbreeding), were found to be strongly associated with competitive release in response to increased resource availability (Dodd *et al.*, 1995). Silvertown *et al* (2002) suggested that drought alleviated interspecific competition that enabled inbreeding and self-compatible plant species to increase their populations. They further postulated that recent evolutionary changes in the mating system of *Festuca rubra,* had occurred. This species is traditionally self-compatible but had acquired a mixed-mating system.

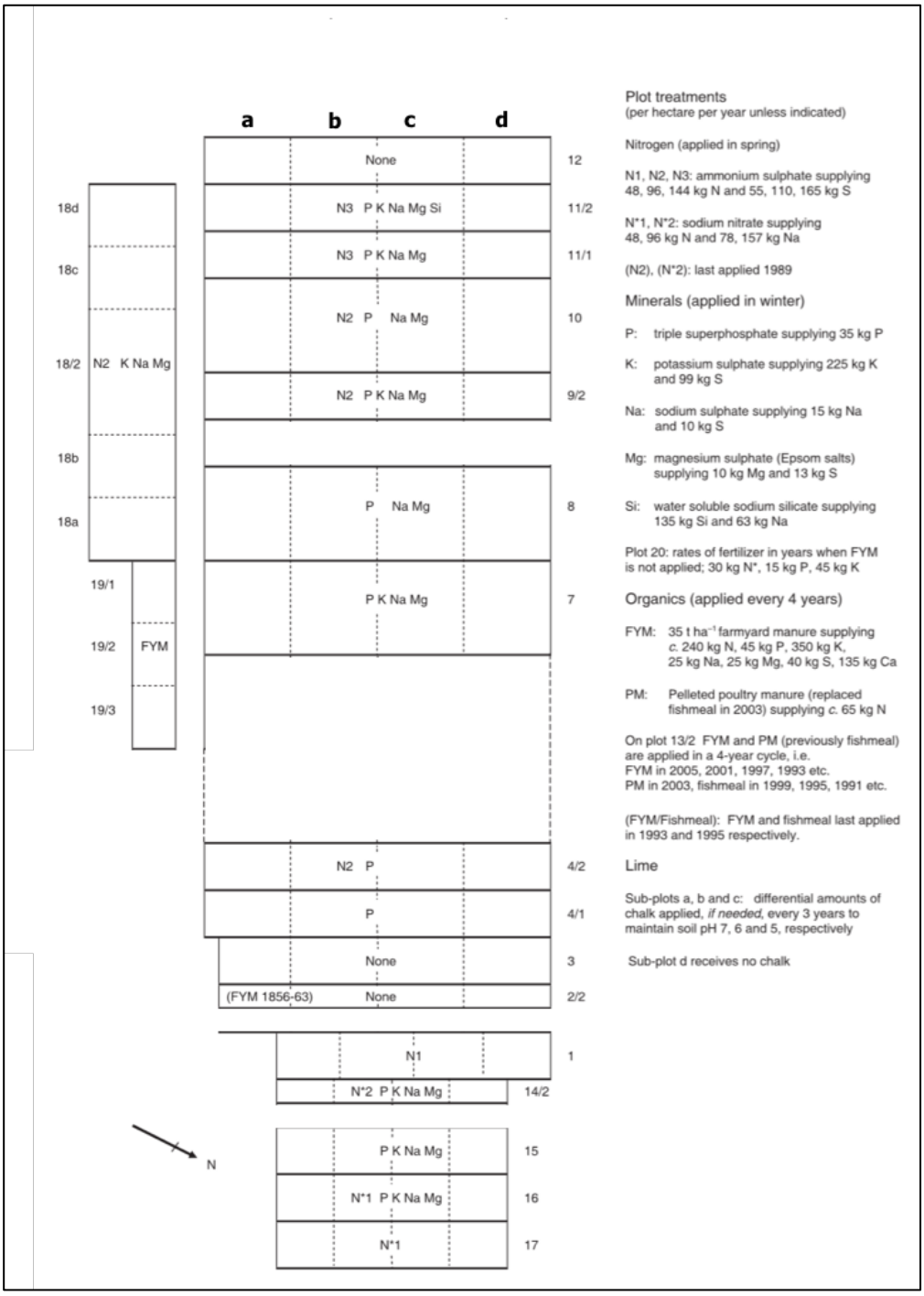

Figure-2.2.1. Plan of the Park Grass Experiment showing fertiliser and liming treatments of those plots under study. "Plot treatments" document the type and application rate of organic and inorganic fertilisers together with the lime regime.

Figure-2.2.2. Aerial photograph of the Park Grass Experiment; 180º rotation of Figure-2.2.1.

2.2.1. An Overview of the Literature from the Park Grass Experiment

The large body of research stemming from the Park Grass Experiment has enriched the study of grassland community ecology and conservation. Research incorporating plant functional traits has been limited and primarily concentrated on the three guilds as representatives of functional groups. Nonetheless, an overview

of the literature would benefit this thesis in providing an examination of the published effects of historical fertiliser and liming treatments on species richness and diversity. The overview situates the research into the Response-Effect Framework by drawing on the species' responses to fertiliser and liming treatments and this effect this has had on ecosystem processes, specifically biomass production (Lavorel and Garnier, 2002).

2.2.1a. Fertiliser and Liming Treatment as an Abiotic Filter

The fundamental notion of abiotic filtering concentrates on the reduction of the local species pool, and plant functional traits, demonstrating convergence (Lavorel and Garnier, 2002). Plant diversity in the Park Grass Experiment was found to have declined to about 30% of its original level during the 135 years of fertilisation and the results of the wider literature suggest differential effects of nitrogen-based (sodium nitrate and ammonium sulphate) and mineral-based (triple superphosphate and potassium sulphate) fertilisers. Crawley *et al* (2005) suggested the size of the species pool was dictated by the rate, variety and combination of fertilisation.

Crawley *et al* (2005) found that nitrogen-based fertilisation at the application rate of 144kg N/ha/yr had the greatest negative impact on species richness in the Park Grass Experiment. Approximately six species were lost upon the application of sodium nitrate and nine lost with ammonium sulphate. The severe effects of ammonium sulphate were first realised in the late 1930's and ammonium sulphate application was linked to decreases in soil mineralisable nitrogen (Richardson, 1938). This was attributed to soil acidification that retarded the decay of organic matter via worms and the virtual absence of nitrifying bacteria. A critical pH value of 4.5 was later confirmed (Silvertown, 1980). Sodium nitrate, on the other hand, increased mineralisable nitrogen through greater yields of nitrogen-rich litter that could be readily decomposed. Liming has also been shown to increase species richness of plots fertilised with ammonium sulphate; for every unit in soil pH increase, two extra species from the legume and miscellaneous functional group were added (Crawley *et al.*, 2005; Storkey *et al.*, 2015). This increase in species richness was found to enhance the resilience of the plot to adverse weather conditions (Silvertown *et al.*, 1994).

Richardson (1938) indicated that heavy application of mineral fertilisers (80 years) did not depress worm populations in the edaphic environment. Plots fertilised with low rates of mineral fertiliser were found to closely match species-rich *Arrhenatherum* grasslands (MG1e) of the National Vegetation Classification typology (Dodd, Silvertown, McConway, *et al.*, 1994). The type and rate of mineral fertiliser application is of paramount importance. Triple superphosphate application was found to lead to the loss of six species on average, however, no significant effects of potassium sulphate were found. The application of phosphorus was found to increase plant species' evenness (Wilson *et al.*, 1996). Finally, substituting mineral fertilisers for organic manures was found to increase richness by two species (Crawley *et al.*, 2005).

As expected, species richness was greatest on plots that had received no extra experimental nutrient input. On these plots, plant species richness is speculated to have returned to 70% of its original after two decades (Storkey *et al.*, 2015). The recovering richness has been attributed to the removal of nitrogen stores via removal of hay from these plots, and the alleviation of interspecific competition. Silvertown *et al* (1994) suggested that abiotic filters, particularly rainfall, operate through biomass as an intermediary that governs the species richness and composition of grass, legumes and miscellaneous species.

2.2.1b. Upscaling to Biomass Yields

Research from the Park Grass Experiment has indicated the direct effects of species richness and indirect influences of fertilisation and environmental variables. Nitrogen fertilisation at the highest rates (96 or 144 kgN/ha/yr) and in combination with triple superphosphate was found to substantially reduce species richness and initiate a botanical shift to tall productive grasses typical of *Lolium perenne* leys (MG7) (Silvertown *et al.*, 1994; Crawley *et al.*, 2005). This functional shift was assumed to be in response to interspecific competition for limiting light resources when nutrient resources are plentiful. A similar shift was reported in response to rainfall whereby non-nitrogen fertilised plots showed increases in the proportion of

grass species. This was attributed to nitrate release in the soil after rainfall (Silvertown *et al.*, 1994). The reduction in species richness, however, was positively correlated with total plot biomass and weakly negatively associated with the stability of hay biomass. Instead, increasing soil alkalinity was found to be strongly related to variability in hay biomass; higher pH resulted in more resilient communities to adverse weather conditions (Dodd, Silvertown, Mcconway, *et al.*, 1994).

2.2.2. The Park Grass Experiment Datasets

The datasets gleaned from the Park Grass Experiment include the fertiliser and liming schedule, botanical survey data and dry matter yields. These were compiled, and the details of the datasets are outlined below.

2.2.2a. Botanical Survey Data

Botanical surveys of the Park Grass Experiment have consisted of visual surveys and the percentage composition of hay. The PARKCOMPIC dataset is a compilation of the most recent, and comprehensive, botanical surveys of the Park Grass Experiment. In the surveys, conducted between 1991 and 2000, six quadrats (50cm x 25cm) were randomly assigned to each plot in early June, immediately before the first cut. The herbage was cut with scissors to ground level and sorted into species in the laboratory. Samples were oven dried at 80^{o}C for 24 hours and the dry mass of each species was determined. Species composition (%) of each plot was determined as well as the total dry mass (gm/0.75m^{2}). This PARKCOMPIC was acquired from the electronic Rothamsted Archive (e-RA) (http://www.era.rothamsted.ac.uk). Annual recordings of species composition (%) per plot were recorded and tabulated as a plot by species matrix. Based on data availability for herbage yield data, 71 plots were selected.

2.2.2ai. Testing for Spatial Autocorrelation in the Botanical Survey Data

Spatial autocorrelation has been defined as the property of random variables taking values of equidistant that are more or less similar than randomly associated. In

essence, there are confounding spatial features across the geographic space. For the Park Grass Experiment, there has been evidence of augmented plant diversity at the periphery of the experimental landscape as predicted by mass effects models (Kunin, 1998). Mass effects are defined as the flow of individuals from areas of high species diversity to unfavourable areas due to spatial proximity. Spatial autocorrelation is an issue because it impairs subsequent statistical tests employed, such as regression and analysis of variance, and typically results in false positive conclusions (Legendre, 1993). It was found that environmental correlates of species distributions were affected by spatial autocorrelation and coefficients were falsely quantified (Dormann, 2007). The statistical analyses of spatial data, therefore, need to incorporate or test for spatial autocorrelation to avoid the pitfalls of spatial pseudoreplication. Spatial autocorrelation can be accounted for by employing a linear mixed-model analysis by incorporating a random effect into the analyses (Bolker *et al.*, 2009). The aim of this section is to test the null hypothesis that plant species abundances of plots and their geographical locations (longitude and latitude) are independent.

Hypothesis testing was executed through the use of a Mantel test. Mantel tests have the ability to associate two dissimilarity matrices in a generalised regression approach (Mantel, 1967). The Mantel test has been widely implemented as a favourable statistical procedure for studying spatial pseudoreplication (Legendre, Fortin and Borcard, 2015). A Mantel test with one thousand replications was performed on two dissimilarity matrices using Euclidean distance and derived from two multivariate matrices: site x plant species matrix; site X geographic location (longitude and latitude). The observed Mantel correlation coefficient was found to be 0.064 with a p-value of 0.062. This result suggests that the Park Grass Experiment is not spatially autocorrelated and therefore fails to reject the null hypothesis that plant species abundances are independent of geographic location. Thus, there is no requirement to employ a linear mixed-model approach in Chapters 4 and 5.

2.2.2b. Herbage Yields Data

Herbage in the Park Grass Experiment is cut and removed twice a year. The first cut was traditionally executed by a scythe, whilst tractor-drawn mowers are used today, in June, with the second cut occurring in autumn (between September and November). Since 1960, yields of dry matter have been estimated from strips cut with a forage harvester (Jenkinson *et al.*, 1994). The PGHAYEQUIV dataset was obtained from e-RA. Yields of dry matter (tonnes/ha) were recorded per plot for the years 1991 to 2000 to match the botanical composition data.

2.2.2c. Treatment Variables

The fertiliser and liming treatments outlined in Figure-2.2.1. were used to create the treatment variables. Together with measures of grass species richness, diversity, evenness, and coverage, as well as soil water pH (taken from "a celebration of 150 years of the Park Grass Experiment"), a complete plot-by-plot dataset, comprising biotic and abiotic variables, was created. The fertiliser, liming and pH variables were fixed for the years 1991-2000, whereas the biotic variables were calculated for each year. Table-2.2.1. summarises this.

Treatment Variable	Data Type
Fertilisation Status	Ordinal
Total Fertiliser Application Rate (kg/ha/yr)	Continuous
Total Nitrogen Fertiliser Application Rate (kg/ha/yr)	Continuous
Ammonium sulphate variety	Ordinal
Sodium nitrate variety	Ordinal
Mineral Addition Status	Ordinal
Total Minerals Application Rate (kg/ha/yr)	Continuous
Triple Superphosphate Application Rate (kg/ha/yr)	Continuous
Potassium Sulphate Application Rate (kg/ha/yr)	Continuous
Sodium Sulphate Application Rate (kg/ha/yr)	Continuous
Magnesium sulphate Application Rate (kg/ha/yr)	Continuous
Water Soluble Sodium Silicate Application Rate (kg/ha/yr)	Continuous

Liming Status	Ordinal
Soil Water pH	Continuous
Grass Species Richness	Continuous
Grass Species' Simpson's Diversity Index	Continuous
Grass Species' Simpson's Evenness Index	Continuous
Grass Species' Coverage (%)	Continuous

Table-2.2.1. Detailing the treatment variables defined by the fertiliser and liming regime of the Park Grass Experiment.

2.3. The British National Vegetation Classification

The National Vegetation Classification was commissioned by the Nature Conservancy Council in 1975 and executed by Professor John Rodwell. A phytosociological approach was adopted, and efforts were focussed on examining the vascular plants, bryophytes and macro-lichen species of vegetation types across Great Britain. Approximately 35,000 samples of vegetation were gathered from natural, semi-natural and major artificial habitats, including the maritime fringe and the tops of the remotest mountains. Short-term leys were a vegetation type that was specifically excluded from the sampling.

Data was recorded using quadrats of differing sizes – dependent on vegetation structure; 2 x 2m quadrats were used for short, herbaceous vegetation and dwarf-shrub, 4 x 4m quadrats for taller and more open communities, sub-shrub heaths and low woodland field layers, 10 x 10m for species-poor or very tall herbaceous vegetation or woodland field layers and dense scrub and 50 x 50m for sparse scrub, and woodland canopy and understorey. A quantitative measure (the Domin scale) was employed to visually assess the aboveground abundance of species. In total, more than 80% of the 10 x 10 km grid squares of Great Britain have been sampled (Rodwell, 1991).

The processing and analysis of the samples produced five published volumes of vegetation types. The volumes describe the vegetation types as in the order of communities, sub-communities, and variants and tabulate summaries of the species

composition of each community and sub-community in the form of "floristic tables" (see Table-2.3.1.). The volumes provide an account of the physiognomy, phenology, and community dynamics as well as biotic and abiotic influences. The amount and quality of this information are incredibly variable.

2.3.1 Selecting Communities and Sub-Communities to Study

In total, 74 lowland grassland communities and sub-communities were selected to study. 1-2% of lowland grasslands are thought to be of high conservation value and the remaining 98% exist as highly fragmented patches (Blackstock *et al.*, 1999). Additionally, lowland grasslands were named in the United Kingdom's Biodiversity Action Plan, which suggested the need to prevent further losses, restore degraded swards to favourable condition and expand patch size of remnant patches (Haines-Young *et al.*, 2000). Lowland grasslands and the ecosystem services they support were reviewed and assessed by Bullock *et al* (2011) and identified as a key target for ecological restoration in the United Kingdom. This rationale, together with publications by the Joint Nature Conservation Committee, and the National Vegetation Classification volumes 2, 3 and 5 informed the selection of the community and sub-community types (Rodwell, 1991, 1992, 2000; Critchley, Burke and Stevens, 2004). Additional communities/sub-communities were included, most notably the sub-communities of *Lolium perenne* leys and related grasslands because of their relationship to the plots of the Park Grass Experiment (Dodd, Silvertown, McConway, *et al.*, 1994). The selected 74 communities span mesotrophic grasslands (MG), calcicolous grasslands (CG), calcifugous grasslands and montane communities (U), mires (M) and shingle, strandline and sand-dunes (SD).

In consultation with Natural England and the Joint Nature Conservation Committee, plot-level species composition data were sought. Unfortunately, this data was unavailable, so instead, the species information contained in the floristic tables for each community was used. These were acquired from the Joint Nature Conservation Committee. The floristic tables summarise a species' frequency, a Species Constancy Value, and its abundance via the Domin Scale. However, the latter was not extracted as the values were incredibly varied for a single species; for

example, *Holcus lanatus* in MG1 had a Domin value between 2 and 8, therefore a corresponding abundance value between 4-75%. The Species Constancy Value, however, was fixed for all species in the communities/sub-communities of interest. This value describes how often a species is encountered in different stands or samples of a vegetation type, regardless of abundance (Rodwell, 1992). Species richness values for each community/sub-community were also obtained from the floristic tables.

2.3.1a. Constructing Hypothetical Communities and Sub-Communities

Species Constancy Value	Frequency	Description	Probability used in Permutation
I	1-20%	Scarce	0.105
II	21-40%	Occasional	0.305
III	41-60%	Frequent	0.505
IV	61-80%	Constant	0.705
V	81-100%	Constant	0.905

Table-2.3.1. Floristic tables assigned species constancy values, their associated frequency ranges and the probabilities used in the permutation of NVC communities/sub-communities.

Using the species constancy and species richness values, 500 plot-level species assemblages were permutated for the 74 communities/sub-communities. A vector of species names and occurrence probabilities (the median probability of the species constancy value- see Table-2.3.1.) for each community/sub-community were submitted for permutation in R. Species were sampled without replacement according to the vector of probability weights to the species richness level (Figure-2.3.1.). Vascular plants, bryophytes, and lichens were initially included in the permutation process. This produced five hundred individual assemblages per community and sub-community represented as presence/absence data. The approach was employed under the assumption that five hundred replications were enough to capture an adequate range of hypothetical communities and sub-communities so that the average represents the true community structure, especially with regards to plant trait values.

2.3.2. Management Groups for NVC Communities/Sub-communities

Management information for each of the 74 communities/sub-communities was extracted from the NVC volumes to create management categories. In total, three management groups were formulated; grazing status covered all of the 74 communities and was divided into three groups; low intensity grazing, grazed and aftermath grazing. Aftermath grazed communities/sub-communities were typically managed for an annual hay cut (harvest after mid-July) and the regrowth grazed in late summer/autumn. The other management groups were specific to mesotrophic grasslands (MG) and were divided according to the application of farmyard manure and improvement status. These are summarised in Table-2.3.2.

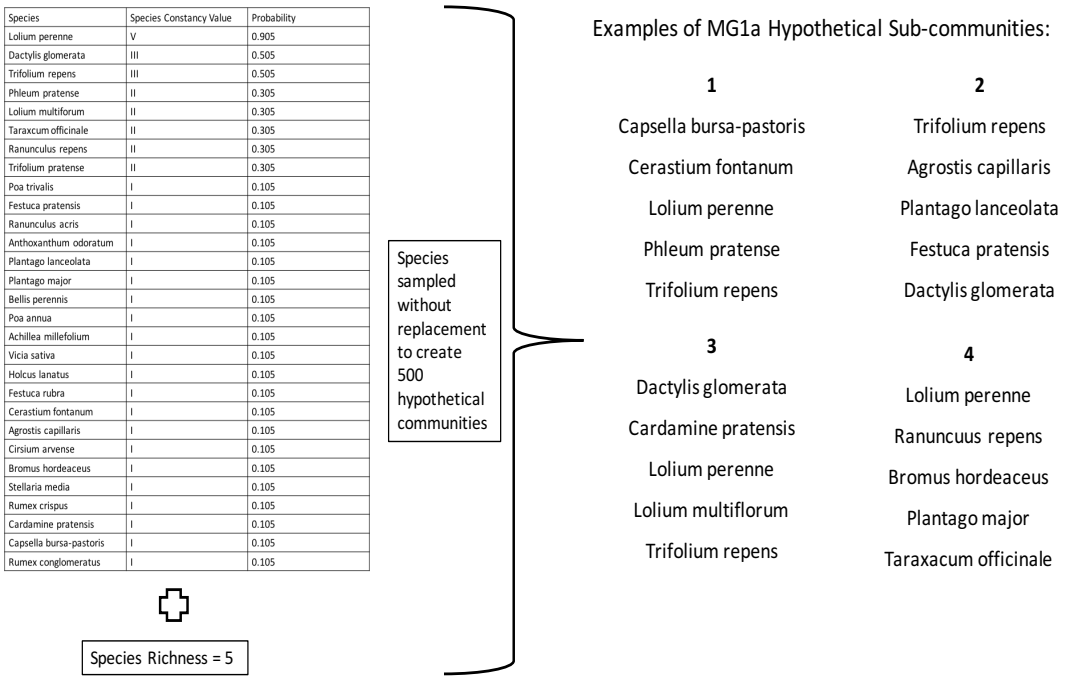

Figure-2.3.1. Illustrative example of the permutation method outlined in 2.3.1a. Four hypothetical communities of MG1a are constructed.

Management Category	Levels	Communities/Sub-communities
Improvement Status	**Unimproved** Grasslands may have received low levels of farmyard manure, but not had sufficient applications of fertiliser or herbicide, or have been intensively grazed or drained.	MG3, MG4, MG5, MG5a, MG5b, MG5c, MG8, MG13
	Semi-improved A transition grassland that has been modified by artificial fertilisers, slurry, intensive grazing, herbicides or drainage.	MG1, MG1a, MG1c, MG1c, MG6, MG6a, MG6b, MG6c, MG9, MG10, MG10a, MG10b, MG10c
	Improved Grasslands that have received heavy grazing, drainage, or application of herbicides, inorganic fertilisers, slurry or high doses of manure. Reseeding may have occurred.	MG7a, MG7b, MG7c, MG7d, MG7e, MG7f
Application of Farmyard Manure	Yes	MG3, MG5, MG5a, MG5b, MG5c, MG13
	No	MG1, MG1a, MG1c, MG1e, MG4, MG6, MG6a, MG6b, MG6c, MG7a, MG7b, MG7c, MG7d, MG7e, MG7f, MG8, MG9, MG10, MG10a, MG10b, MG10c

Table-2.3.2. Details of the management groups defined for mesotrophic grasslands of the National Vegetation Classification.

2.4. The North Wyke Farm Platform (NWFP)

The NWFP is located at Rothamsted Research, North Wyke Farm in the South West of England. The platform is based on clay-rich sub-soils beneath the sub-surface horizon and is divided into three individual farmlets, approximately 21 hectares each, consisting of five catchments each (Figure-2.4.1.). Each catchment is hydrologically isolated via a combination of topography and a network of 9.2km drains constructed around the perimeters of each catchment. Catchments were allocated to farmlets based on the physical properties of the North Wyke site, spatial connectivity within a farmlet, historical farming practice and farm/research operational requirements. Each farmlet is managed using alternative approaches to test the hypothesis that grasslands can be designed and managed to deliver maximum sustainable production (product/unit area/unit animal) with reduced impacts on the environment (Pilgrim *et al.*, 2010). The three approaches were implemented in April 2013 and matured in late 2015 (Orr *et al.*, 2016). They are postulated to be innovative solutions for the sustainable intensification of livestock production systems (Hawkins, 2016a). The main treatments are:

1. Blue Farmlet: increased use of legumes through seeding (drill seeding) of legumes (AberHerald – *Trifolium repens*) and grass mixtures (AberMagic – *Lolium perenne*) to replace inorganic fertilisers with biological fixation.
2. Red Farmlet: the planned reseeding, via drill seeding, of innovative varieties with desirable traits, such as high sugar and deep rooting grasses. The cultivar Prior – *Festulolium loliaceum* – is the main candidate for the reseeding activities.
3. Green Farmlet: sward improvement of extant grassland through the use of artificial fertilisers.

The data portal for the North Wyke Farm Platform was only established in March 2016 and details the baseline surveys of the platform conducted in 2013. The baseline surveys have been suggested to illustrate the utility of the North Wyke Farm Platform and with expansion can provide the means to develop broader assessments of livestock production systems, particularly in the realms of life cycle assessments and environmental accounting reports, which could consider carcass

and meat quality with environmental metrics (Orr *et al.*, 2016). This will inevitably facilitate a holistic examination of food production systems that stems beyond livestock density and product yields per hectare (Orr *et al.*, 2016). Using the Soil-Plant Atmosphere Continuum System model, long-term responses of the grassland systems to the three management scenarios were investigated at the field-scale (Wu *et al.*, 2016). Wu *et al* (2016) demonstrated that the management activities of the blue and red farmlets would be sustainable under the current scenarios. In fact, the catchments reseeded with legumes and grass mixtures could increase carbon fixation and nitrogen offtake and potentially result in greater livestock output. The red farmlet is thought to benefit the carbon sequestration and reduce soil surface runoff, and remain stable during weather extremes (Wu *et al.*, 2016).

Recent surveys of the North Wyke Farm Platform have sampled the climate and environmental features, edaphic conditions, the botanical composition, herbage and silage cuts, as well as livestock (cow, sheep, and lamb) records. Those used in this thesis are described in the following sections.

2.4.1. Climate and Edaphic Features

Water quality in each of the catchments is monitored using flow bypass cells, which are an amalgamation of three instruments that take measurements every fifteen minutes. The parameters measured include dissolved oxygen, turbidity, specific conductivity, temperature, pH, ammonium (NH_4^+), ammonia (NH_3), combined nitrate-N and nitrite-N, dissolved organic carbon (DOC) and total phosphorus (TP). Additionally, each of the 15 catchments has a soil moisture station (SMS) sites at a central location that measures soil moisture through capacitance at depths of 10, 20 and 20cm, soil temperature at 15cm and precipitation measured by a rain gauge (Hawkins, 2016b). Annual data (2013) for the climate and environmental variables, shown in Table-2.3.2. were obtained from the NWFM data portal (https://nwfp.rothamsted.ac.uk). The data summarised the climate and environmental measurements taken every fifteen minutes in 2013 for each catchment.

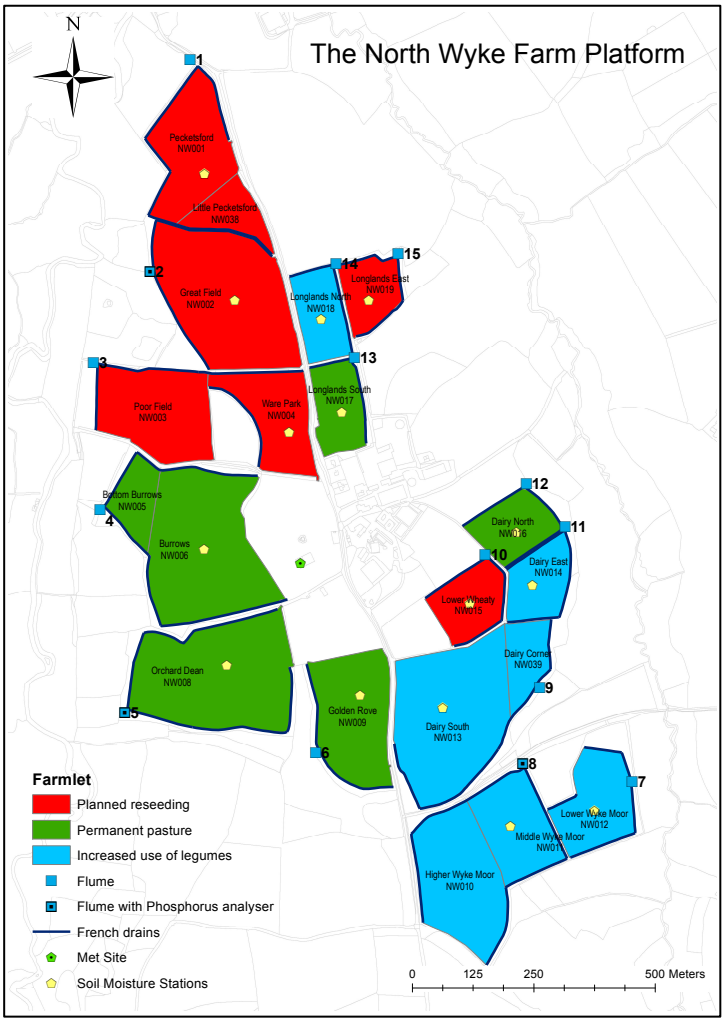

Figure-2.4.1. Map of the North Wyke Farm Platform showing farmlets as of 2015.

2.4.2. Botanical Survey

The North Wyke Farm Platform was surveyed between 22/07/2013 and 07/08/2013 for a botanical composition of eleven catchments, and this was acquired from the North Wyke Farm Platform data portal. There were 293 sampling locations using quadrats (0.25m^2) with species recorded according to the Domin Scale (Harris, 2016). The Domin Scale is criticised for having a non-linear relationship with species abundance and upon averaging tends to under-estimate the true value of species abundance. A transformation of the Domin Scale into Domin 2.6 was proposed to resolve these (Currall, 1987). This next section compares the Domin 2.6 scale with the original scale and analyses the results of a spatial autocorrelation analysis.

2.4.2a. Transformation of the Domin Scale

The original Domin scale is restricted to ordinal abundance estimates. Ordinal estimates are criticised for limiting arithmetic operations because they represent a non-linear scale and thus the differences between Domin values are not consistent. For example, the difference between Domin scores of 1 and 2 (both approximated at <4%) is clearly not identical to that between 5 (16.5%) and 6 (29%). This non-linear scale under-estimates the true value of cover upon averaging (Currall, 1987). The commonest suggestion for overcoming the limitations of ordinal data is to introduce a transformation which is claimed to improve the numerical properties of cover-abundance data. Bannister (1966) considered the Domin scale to be an amalgamation of percentage cover in the quadrat. However, Bannister's approach was criticised for not being accurate as it poorly transformed Domin scores of 2 or less. Currall (1987) proposed the Domin 2.6 scale. It was found that Domin 2.6 demonstrated a very good fit to the relationship between percentage cover and Domin score through explaining 98.91% of the total variation (Currall, 1987). Further support for the Domin 2.6 scale manifests itself in its wide implementation as an appropriate transformation (Dale, 1989; Hill, 1989; Critchley *et al.*, 2002). The rationale of Currall and the use of Domin 2.6 in the literature demonstrate the adequacy of employing this transformation to the botanical survey data of the North Wyke Farm Platform.

$$Abundance = \left(\frac{Domin\ Score^{2.6}}{4}\right)\Big/_{100}$$

Domin Score	Cover	Abundance
10	91-100%	0.9953
9	76-90%	0.7568
8	51-75%	0.5572
7	34-50%	0.3937
6	25-33%	0.2637
5	11-22%	0.1642
4	4-10%	0.0919

3	<4% (many individuals)	0.0435
2	<4% (several individuals)	0.0152
1	<4% (few individuals)	0.0025

Table-2.3.1. Detailing the transformed Domin Scale into cover-abundance data and then into the Domin 2.6.

The 293 sampling locations were assigned a unique SP_ID at the time of sampling. These SP_IDs were consistent across the surveys of herbage and soil in 2013, and therefore were used to match botanical composition data with the measures of edaphic variables and herbage survey. The details of these surveys and their associated measures are outlined in the coming section.

2.4.2b. Spatial Autocorrelation in the North Wyke Farm Platform

Spatial pseudoreplication was investigated in the baseline botanical survey data from 2013. A Mantel test was conducted based on two Euclidean distance dissimilarity matrices, which were constructed from a catchment x species abundance and site x geographic position (easting and northing) tables. Similar to the Park Grass Experiment, this aimed to investigate independence of individual catchments. The Mantel test was replicated 1000 times and found an insignificant (p-value = 0.06) observed Mantel correlation coefficient of 0.008. This result suggests that the North Wyke Farm Platform is not spatially autocorrelated and therefore fails to reject the null hypothesis that plant species abundances are independent of geographic location. Thus, a linear mixed-model approach does not need to be executed in chapter 5.

2.4.3. Ecosystem Services

Ecosystem services can be viewed as latent variables meaning they are not directly observed or measured but rather inferred from other variables. The use of indicator variables, typically representing ecosystem processes, are commonly used to ascertain ecosystem services' provision. Pakeman (2014) operationalised food production via livestock production through a single measure of livestock units per hectare per year. The analyses of this thesis reduce the broad ecosystem services

of biomass production and animal production into observable variables that correspond to latent measures of quantity and quality outlined in 2.3.3a. and 2.3.3b.

2.4.3a. Biomass Production ≡ Quantity and Quality of Forage Material

A plant nutrients (herbage) survey was conducted in the summer of 2013 on a mixture of 25m and 50m sampling grid locations. All 15 catchments were sampled for the parameters summarised in Table-2.3.2. (Harris, 2016).The data was obtained from the NWFP data portal.

These parameters are used in this thesis as different measures of forage material. Average sward height was assumed to be a quantitative measure of green biomass, whereas Total-N and Total-C were quality measures. Quality, in this respect, was defined according to forage quality characteristics. Total-N is a widely recognised forage quality trait and is directly related to forage protein content, which is essential to the rate of gut passage of ingested material in ruminants to avoid bulk limitation (Adler *et al.*, 2004). Furthermore, Total-N of herbage has been linked to the palatability of herbage and, thus serves a function in livestock foraging behaviour (Lloyd *et al.*, 2010). Conversely, Total-C has negative associations with forage quality; both palatability and digestibility. High levels of Total-C are suggested to be an indicator of high lignin concentrations and neutral detergent and acid detergence fibres; all of which impede herbage digestibility and limit livestock production (Ansquer *et al.*, 2009b; Pakeman, 2014b).

Variables	**Parameters Measured**
Climate and Environmental	Ammonium/Ammonia (mg/l), Nitrite and Nitrate (mg/l), soil pH, Precipitation (mm), Soil Moisture @ 10cm, 20cm and 30cm (%) and Soil Temperature @ 15cm (^{o}C).
Botanical Composition	Domin scale converted to Domin 2.6.
Ecosystem Services	**Biomass Production**

	Total-N (% of Dry Matter), Total-C (% of DM) and Average Sward Height (cm). **Food Production** Cattle: Rate of Weight Gain (kg/day), Abattoir Premiums and Penalties (pence/kg), Final Live Weight (kg), Cold Carcass Weight (kg), Dead Weight Value (pence/kg) and Cow Stocking Units (LU/ha/yr). Lamb: Rate of Weight Gain (kg/day), Abattoir Premiums and Penalties (pence/kg), Final Live Weight (kg), Cold Carcass Weight (kg), Dead Weight Value (pence/kg) and Lamb Stocking Units (LU/ha/yr). Total: Rate of Weight Gain (kg/day), Abattoir Premiums and Penalties (pence/kg), Final Live Weight (kg), Cold Carcass Weight (kg), Dead Weight Value (pence/kg) and Lamb Stocking Units (LU/ha/yr). Total Livestock Stocking Unit (LU/ha/yr).

Table-2.3.2. Detailing the variable classes and associated measures gleaned from the North Wyke Platform database.

2.4.3b. Food Production ≡ Rate, Quantity, and Quality of Livestock Production

From 2011, the NWFP was continuously stocked with cattle and sheep. The cattle are a spring-calving suckler herd of Hereford X Friesian cows and the sheep are a March-lambing flock of Suffolk X Mule ewes crossed with Texel or Charollais rams. In the grazing season of 2013, the livestock were allocated to the green, blue or red farmlets (Orr and Griffith, 2016).

The data available, and obtained from the NWFP data portal, on the livestock of the NWFP included; location data recorded throughout 2013, weight data recorded at irregular intervals in 2013, together with lamb and cattle sales data that documented final live weight (kg), cold carcase weight (kg), conformation, fat class

and dead weight price (pence/kg). Using this recorded information indicators of livestock production corresponding to rate, quantity and quality were defined.

The rate of weight gain was calculated for cattle and lambs. This was achieved by tracking the locations of individuals, via their official tags, throughout 2013 and matching this data with the weight recordings. In total, 590 individuals (333 cattle, 257 lambs) were tracked and measurements of the rate of weight gain (kg/day) recorded. This data was then matched with the final live weight (kg), cold carcass weight (kg) and dead weight price (pence/kg). An additional, parameter of abattoir premiums and penalties (pence/kg) was also calculated (Orr and Griffith, 2016).

Abattoir premiums and penalties were selected as a measure of livestock meat quality. Beef and lamb carcases are graded according to their conformation and fat class. These grades are dictated by the abattoirs according to demand of the market. According to the English Beef and Lamb Executive (2012), the current grading system for carcases in the UK and Europe uses the EUROP classification for the conformation and a numeric assessment for fatness (classes 1-5). Using the example pricing grids published in in the Beef BRP Manual 2 and the Sheep BRP Manual 1, estimation of abattoir premiums and penalties (pence/kg) were calculated for each slaughtered individual. The examples of the pricing grids for an abattoir are shown in Table-2.4.3. and 2.4.4.

Livestock units are traditionally implemented to determine overall grazing pressure. However, Pakeman (2014) used livestock units (LU/ha/yr) as a measure of secondary production (herbivore productivity) to investigate its relationship with functional diversity and leaf dry matter content (LDMC). This measure of secondary production was also adopted in this way. Livestock units were calculated for each catchment and livestock variety (cattle, sheep, and lambs). Total livestock units were also calculated. The existing method of describing types of livestock in terms of units is defined by the European Commission (EC), which has defined livestock equivalents based off feed intake and live weight. These equivalents were used to calculate the livestock units at the catchment level: sheep (15 catchments), lamb

(11 catchments) and cattle (6 catchments). The European Commission equivalence factors used were: sheep and lambs (0.15) and cattle (1).

$$\mathrm{LU} = \frac{(Count\ x\ Equivalence)}{Area} X \frac{Number\ of\ Days}{365}$$

	1	*2*	*3*	*4L*	*4H*	*5L*	*5H*
E	-10	+15	+20	+20	+15	-10	R
U+	-10	+15	+15	+15	+8	-15	R
-U	-15	+5	+5	+5	0	-20	R
R	-20	0	0	0	-5	-25	R
O+	-30	-10	-10	-10	-15	-30	R
-O	-40	-30	-20	-20	-30	-40	R
P+	-50	-40	-40	-40	-50	-60	R
-P	-70	-50	-50	-50	-70	R	R

Table-2.4.3. Example Cattle abattoir premiums and penalties based on the EUROP classification.

	1	*2*	*3L*	*3H*	*4L*	*4H*	*5*
E	0	+15	+15	0	-25	-45	-80
U	0	+8	+8	0	-25	-45	-80
R	-5	0	0	-5	-25	-45	-80
O	-20	-5	-5	-15	-30	-55	-80
P	-70/100	-70/100	-70/100	-70/100	-70/100	-70/100	-70/100

Table-2.4.4. Example Lamb abattoir premiums and penalties based on the EUROP classification.

2.5. Trait Databases

2.5.1. Introduction to the TRY Initiative

The TRY initiative is a collection of 93 trait databases that have more than five million trait records for 1100 traits and 2.6 million individual plants spanning

100,000 plant species. Each and every trait is standardised, and quality checked (outlier analysis and duplicate identification). The initiative was established to provide a comprehensive web-archive of functional biodiversity of plants to facilitate and promote trait-based approaches aiming to analyse the dynamics and predict the ecosystem consequence of plant biodiversity loss (Kattge *et al.*, 2011).

The TRY data portal (https://www.try-db.org) focuses on 52 groups of traits characterising the vegetative and regenerative stages of the plant life cycle (growth, reproduction, dispersal, establishment, and persistence). Trait data covers both qualitative and quantitative measures of all aspects of the plant life cycle. Recordings for qualitative measures span more species than those of quantitative measures but they tend to be lower in interspecific variation than continuous plant traits, therefore, quantitative plant traits were used exclusively throughout this thesis (Weiher *et al.*, 1999).

The quantitative traits found to cover 5000-20,000 species were seed mass, plant height, wood density, leaf size, leaf nitrogen content and specific leaf area (SLA). In contrast, plant root trait data is incredibly sparse and covers less than 0.05% of the vascular plant species. This is due to the laborious task of quantifying root anatomy from microscopic cross-sections (Kattge *et al.*, 2011). Eleven quantitative plant traits were originally selected due to their known relationships with plant community assembly and the ecosystem services of interest in this thesis; primary production and secondary production (livestock production). These eleven traits and number of species covered by the TRY database are shown in Table-2.5.1.

Trait (Units)	**Number of Species**	**% of Species**
Plant Height (m)	26837	99.16
Leaf dry matter content (g/g)	4388	91.98
Leaf nitrogen content per leaf dry mass (mg/g)	10149	63.25

Leaf carbon content per leaf dry mass: Leaf carbon content per leaf dry mass	4496	54.79
Leaf phosphorus content per leaf dry mass (mg/g)	5882	40.75
Leaf photosynthesis rate per leaf area (µmol/m/s)	2192	21.87
Leaf thickness (mm)	5324	91.98
Specific Leaf Area (mm^2/mg)	11991	95.10
Seed dry mass (mg)	26095	93.31
Seed Oil Content (% weight of seed mass)	2923	29.16
Seed protein content (% weight of seed mass)	2343	20.27

Table-2.5.1. Detailing the plant traits requested from the TRY database, together with the number of species covered in the database and % of species covered in this thesis.

A compiled list of the vascular plant species covering the entirety of this thesis was created. Species' names were examined across the datasets to ensure consistency in taxonomy – basionyms and nomenclatural synonyms were checked for each species using the International Plant Names Index (2012). In this initial list of 480 species, phanerophytes were removed and the plant traits were requested from the TRY portal for the remaining 449 vascular plant species. Mean trait values for each species were calculated from the requested data as mean values from functional databases are assumed to be robust and interspecific differences are surmised to be more important than intraspecific variation (Cornwell and Ackerly, 2009; Albert *et al.*, 2010). The percentage of species recordings for the eleven traits are shown in Table-2.5.1. The plant traits that failed to cover over 50% of plant species were removed; leaf phosphorus content, leaf photosynthesis rate, soil oil content and seed protein content.

Inadequacies in plant trait quantification have been documented and authors have made various recommendations to resolve this problem. Widespread analysis of underrepresented plant traits, such as seed protein content, has been postulated but has been countered given the labour and financial expense. Garnier *et al* (2017) suggested that the lack of trait data can be resolved semantically by creating a universal standard for acquiring, organising and describing plant trait data and subsequently proposed the Thesaurus of Plant Characteristics. The implications of this, however, have yet to be examined but is thought to increase the accessibility to plant trait data (Garnier *et al.*, 2017). Another avenue has taken into account of phylogeny under the assumption that plant trait measurements are conserved within lineages and therefore can be adequately predicted (Penone *et al.*, 2014). Hierarchical probabilistic matrix factorisation modelling was found to improve the accuracy for predicting missing plant trait values (Shan *et al.*, 2012). This imputation method, however, is dependent on a strong phylogenetic signal for the traits in question. Moreover, plant trait conservatism is thought to confound the conclusions of plant trait research as the influence of phylogeny on plant trait analyses cannot be unravelled. Swenson (2014), however, suggested that many traits are not phylogenetically conserved and so there isn't a necessity to account for conservatism. Swenson's rationale was also assumed in this thesis.

2.5.2. Imputation of Missing Trait Values

Research has indicated that only two traits of the 1100 traits in TRY are measured for a single species. The incompleteness of the trait databases has long been an issue for Functional Ecology due to the limitations in time and monetary resources to comprehensively measure trait values for every plant species on earth. Plant traits of temperate ecosystems are the most categorised but deficiencies can still restrict analyses (Schrodt *et al.*, 2015). A great concern for this thesis was the impact of missing data on the indices of the Biomass-Ratio Hypothesis and Functional Diversity Hypothesis. For community-weighted means, known trait values are recommended to account for at least 80% of the floristic biomass or abundance, whereas functional diversity metrics are highly sensitive to minute omission of species (Pakeman and Quested, 2007; Pakeman, 2014a). A full range of techniques to cope with missing trait values has been suggested; from removing or ignoring missing species values to a hierarchical Bayesian extension of a

probabilistic matrix factorisation approach and phylogenetic techniques (Schrodt *et al.*, 2015). The latter, however, has a serious consequence of reducing statistical power and jeopardises the conclusions of said research (Nakagawa and Freckleton, 2008). The development of sound imputation methods has, thus, proceeded in recent years.

Currently, the imputation methods rely on the notion that missing trait values are random and given a suite of other traits, phylogenetic, functional and structural trade-offs and synergies between traits can be deduced and translated (Rubin, 1976). Penone *et al* (2014) reviewed the relative performance of four single and multivariate imputation methods; k-Nearest Neighbour, multi-variate imputation by chained equations, a random forest technique and an approach based on a maximum likelihood that uses phylogenetic information. The results provided support for the use of all four methods and were reliable in estimating 60% of missing values, and found that allometric relationships between plant traits were preserved (Penone *et al.*, 2014). Penone *et al* (2014) recommended specifying a tuning parameter in the selection of the number of neighbours used in the k-Nearest Neighbour approach and stresses the difficulty to determine theoretically. A resolution to this has manifested in the dissimilarity imputation method.

Missing values of T_j of species S_i are imputed using Gower's dissimilarity coefficient between S_i and the other species based on other trait values. Species showing high similarity, functionally proximal in trait profiles and allometric relationships, are selected and the median value of T_j computed and used to impute the missing value T_{ij}. A threshold value of ≥ 0.05 for the Gower's dissimilarity coefficient was used to discriminate neighbouring species. This approach has been adopted in the literature and was the imputation method implemented in this thesis for estimation leaf nitrogen content and leaf carbon:nitrogen ratios (Taugourdeau *et al.*, 2014).

2.6. Illustrative Summary of Datasets and Methods

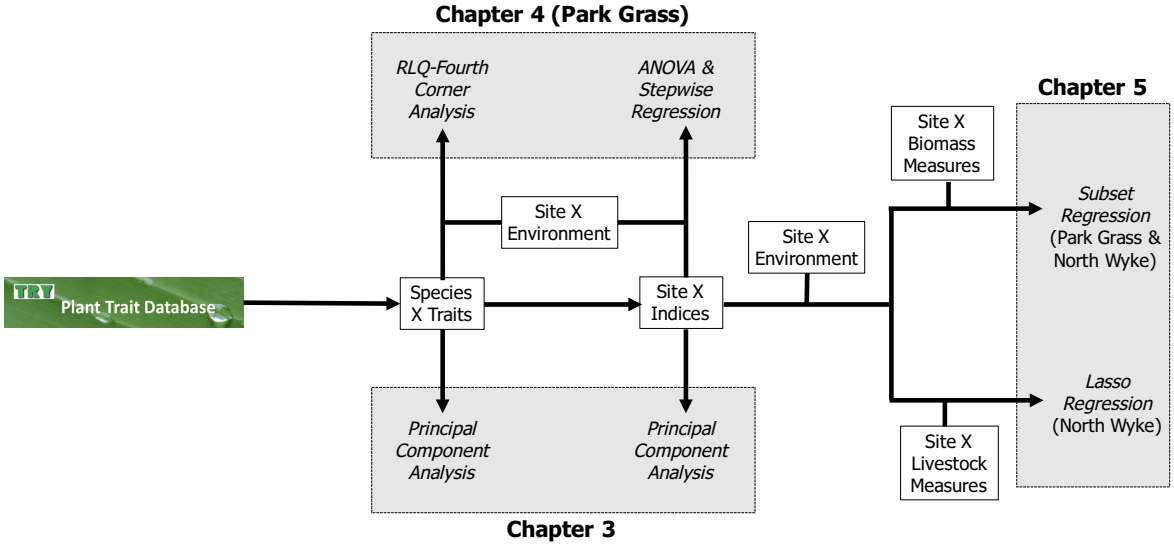

Figure-2.5.1: Illustrative summary of the constructed data matrices (sold lined rectangles) and their corresponding statistical analyses (italicised) situated in each chapter (grey shaded rectangles).

Assessing Ecological Axes of Specialisation and Redundancy in Functional Diversity Indices

3.1 Introduction

Functional plant ecology is premised on the belief that plant traits can be used as general tools to describe, analyse and predict growth, survival and reproduction of plant communities across biomes (Garnier *et al.*, 2007). Plant ecology typologies have been continually formulated to categorise plant species according to their unique ecologies (Westoby, 1998). Scholarly attention has increased in the definition of plant ecology strategy schemes, but despite this there is a lack of a universally agreed scheme. Those that exist have primarily centred on three ecological concepts:

1. Fundamental/Realised niches: traditionally based on the *ad hoc* classification of plant species according to environmental gradients, such as shade and grazing tolerance/avoidance schemes and Grime's CSR triangle (Givnish, 1988; Westoby, 1998).
2. Biogeography: focusses on categorising plant species based on qualitative plant traits of physiognomic and phenological significance, for example, Raunkiaer's life-form scheme (Westoby, 1998).
3. Ecological selection: based on the types of ecological opportunities exploited by an individual, species or population, such as the r-k spectrum (Westoby, 1998).

In consideration of these concepts, ecological strategies have focussed on the *a priori* formulation of functional groups. The use of discrete groupings has been heavily criticised by many authors and Reich *et al* (2003) found typologies based on qualitative plant traits did not effectively discriminate herbaceous species. Continuous quantitative traits, however, were found to sort herbaceous species according to trait combinations. This comparative view of species assumes that

plant trait relationships are the result of adaptive evolution, which has shaped interspecific variation and speciation (Messier, McGill and Lechowicz, 2010). It is assumed these interspecific differences in plant traits transgress spatial scales and the identification of a core list of plant traits will aid the application of dynamic global vegetation models, community assembly rules and the modelling of ecosystem processes and services (Weiher *et al.*, 1999; Lavorel and Garnier, 2002). Analysing the dimensions of trait variation and their independence has been central to constructing a core list of plant traits and assorting them into meaningful syndromes. Individuals are collectively described by their species mean functional trait values under the assumption that interspecific variation is greater than intraspecific variation. Albert *et al* (2010) supported this notion and found that individual -and population-level analyses resulted in the same general trait space structure and leading axes of specialisation documented at the species-level.

3.1.1. The Leaf-Height-Seed Scheme

The Leaf-Height-Seed (LHS) scheme published by Westoby (1998) adapted the CSR triangle incorporating the plant traits of specific leaf area, plant height and seed mass. These were assumed to simplify and represent the core ecological strategies that Grime described. The LHS added a further dimension to Grime's original theory; the viability of individuals at high S and high R strategies. The original LHS scheme assumed independency of these three traits and has inspired decades of research to elicit the ecological axes of specialisation and their associated ecological strategies. Studies emerging from Grime's original conception have expanded the trait profiles of the CSR strategies. The partitioning of the LHS scheme has resulted in three individual spectra/axes, which are mostly agreed upon (Peter J. Wilson, Thompson and Hodgson, 1999).

3.1.2. Leaf Economics Spectrum

Since the recognition of foliar traits by Westoby (1998), research has found universal and convincing evidence of a single leaf dimension with consistent trait relationships at different spatial scales (growth form, biome and climatic region) (Wright *et al.*, 2004). This spectrum was named the Leaf Economics Spectrum. Leaf

mass per area and its inverse specific leaf area were the first plant traits to feature and since has been associated with the establishment and persistence phases of community dynamics (Westoby *et al.*, 2002). Evidence for the establishment via increased seedling growth and persistence through the ability to respond to disturbances caused the development of an ecological strategy scheme describing the dichotomy of exploitative and conservative species. This has been suggested to be a universal scheme that transgresses growth forms and geographic barriers. Freschet *et al* (2010) found that exploitative and conservative strategies were evident in aquatic plant species and the subarctic ecosystem.

Exploratory analyses have further shed light on the foliar synergies and trade-offs typical of the Leaf Economics Spectrum, and these have been related to morphological, biochemical and metabolic characteristics (Table-3.1.1.). In relation to exploitative and conservative strategies, Craine *et al* (2002) found that nitrogen-conservativism is manifested as investment in morphologically tough, metabolically slow and biochemically impoverished leaves. Specific leaf area was suggested to be the most useful indicator of the Leaf Economics Strategy as it encapsulates many elements. Increased specific leaf area is associated with reduced construction costs per unit leaf area such that leaf physical strength, leaf thickness and leaf dry matter also decreased (Westoby *et al.*, 2002). These morphological and biochemical distinctions are characteristic of the exploitative strategy.

Metabolically-speaking, specific leaf area is positively correlated with relative growth and photosynthesis (Weiher *et al.*, 1999). Thinner leaves are suggested to be conducive to stomatal conductance that facilitates carbon dioxide diffusion to chloroplasts supporting photosynthesis. Additionally, the partitioning of leaf nitrogen is thought to be invested into metabolic entities, predominantly the enzyme Rubisco (Westoby *et al.*, 2002; Wright *et al.*, 2005).

The cornerstone of the Leaf Economics Spectrum has been deliberated. Early research highlighted specific leaf area as the lead contender, but leaf dry matter content was also found to be a better discriminator of exploitative and conservative

species (Peter J. Wilson, Thompson and Hodgson, 1999). Furthermore, Westoby *et al* (2002) suggested that leaf thickness, as a proxy for leaf lifespan, displayed an interspecific variability that was 100 times greater than intraspecific variation. Finally, leaf nitrogen content was suggested as a single representative of the Leaf Economics Spectrum (Lavorel and Grigulis, 2012). What can be gleaned from the literature is the importance of specific leaf area, leaf dry matter content, leaf thickness and leaf nitrogen content to the Leaf Economics Spectrum and these traits should be integral to plant trait-based investigations.

	Relationship with Specific Leaf Area	
Morphological	Leaf thickness	↓
Metabolic	Leaf photosynthesis rate per leaf dry mass	↑
	Leaf dark respiration rate per leaf dry mass	↑
	Plant relative growth rate	↑
	Leaf life span	↓
	Stomatal conductance	↑
Biochemical	Leaf nitrogen content per leaf dry mass	↑
	Leaf C:N content per leaf dry mass	↓
	Leaf cellulose content per leaf dry mass	↓
	Leaf dry matter content	↓
	Micronutrient content per leaf dry mass	↑
	Lignin content per leaf dry mass	↓

Table-3.1.1. Relationships of morphological, metabolic and biochemical plant traits with specific leaf area documented with reference to the Leaf Economics Spectrum. Their associations are in comparison to Specific Leaf Area.

3.1.3. Plant Height Axis

Plant height has been ubiquitously adopted as a classic representation of an axis that can be understood in the context of game theory (Westoby *et al.*, 2002). Strategies associated with plant height have been linked to a plants' ability to persist through the hard traits of fecundity and competitiveness (Weiher *et al.*, 1999). Increased plant height is suggested to confer greater acquisition of light, metabolic activity and biomass. This increases the mechanical costs to support stem tissues, reduce breakages and reduces the efficiency of water transpiration to distant leaves (Lavorel and Grigulis, 2012). Lavorel and Grigulis (2012) found that the axis associated with plant height is independent to the Leaf Economics Spectrum.

3.1.4. Seed Mass Axis

Seed mass has been linked to the hard traits relating to a species' dispersal, establishment and persistence (Weiher *et al.*, 1999). Seed mass is commonly used as a quantitative proxy for traditionally qualitative traits, such as dispersal mode/distance and seedbank longevity. Lighter seeds are suggested to disperse further, germinate and establish fast, and mature plants produce large outputs of seeds that have a short residence time in the seedbank. Westoby *et al* (2002) found that seedlings from larger-seeded species had greater investment in resource reserves that supported respiration longer under carbon deficit and was coined the cotyledon functional morphology hypothesis. Grime *et al* (1997) had already subscribed to this notion suggesting that maternal allocation in seeds dictated the number and survivability of seedlings in the regeneration of populations.

3.1.5. Ecological Axes of Specialisation and Grime's CSR

Support for these fundamental axes has been paramount and attempts to further reduce them have been documented. The research of Sandra Díaz and her colleagues has been at the forefront of defining global ecological axes of specialisation. Her first global study ordinated 640 plant species from three continents on the basis of twelve traits and confirmed the independence of the Leaf Economic Spectrum and the Plant Height Axis together with a further axis of life

history and clonality (Díaz *et al.*, 2004). The results demonstrated that major axes of specialisation could be broadly applied to contrasting flora, environmentts and growth forms. The findings of Díaz et al (2015) shed further light on the global patterning of plant traits. A six-dimensional trait space was found to be adequately explained by the first two planes. The first dimension aligned with the Leaf Economics Spectrum, and the second condensed the Plant Height and Seed Axis into a single dimension (the Size Axis) (Díaz *et al.*, 2015). These findings postulate that ecological axes of specialisation are prominent at the global scale and appear to be phylogenetically and geographically conserved. The formation a single Size Axis together with the Leaf Economics Spectrum has simplified successive approaches in functional ecology, however, there is considerable doubt in the consistency of plant trait variation and independence across different ecological scales. Messier, McGill and Lechnowicz (2010) reported that interspecific variation was found to be equal to intraspecific variation at the leaf-, tree-, strata-, species- and site-level. However, both Wright *et al* (2005) and Garnier *et al* (2007) urged against the universal implementation of plant trait syndromes.

The list and number of plant traits and dimensions should be open-ended (Westoby *et al.*, 2002). The pursuit of plant dimensions has focussed on minimalism because of the assumed intrinsic redundancy of plant traits (correlated) (Lavorel *et al.*, 2002). In reality, the list and number of plant traits is driven by the subjectivity of the investigator depending on the aims of the research; the local and regional geography and the investigation of generalizable relationships with ecosystem processes and services (Lavorel and Grigulis, 2012). Westoby *et al* (2002), nevertheless, urged the inclusion of three core traits: leaf mass per area (or specific leaf area), seed mass and plant height. However, Laughlin (2014) found that increasing dimensionality beyond 4 plant traits to nine accurately discriminated species identities, which increased the predictability of models of community assembly. Additionally, scholars have indicated that dimensionality may be ecosystem- or growth form- specific in that ecological strategies can be specific to the systems and types they are defined in, for example, a four-dimensional space can adequately explain trait variation in woody species (Pierce *et al.*, 2013). More detailed understanding of ecosystem-specific trait variation will improve the accuracy of mechanistic models predicting the delivery of ecosystem processes and

services (Bernhardt-Römermann *et al.*, 2008). Pierce *et al* (2013) postulated that a four-dimensional space for woody species could model ecological changes in response to anthropogenic disturbances, such as deforestation and nitrogen deposition.

The dimensionality and redundancy among plant traits needs deeper examination across different spatial scales and to integrate different plant organs. The correlations exposed, thus far, have been shown to hold true over entire floras and for contrasting environments, however the consistency of these relationships within ecosystems or communities has been challenged (Lavorel and Garnier, 2002). To optimise accuracy, functional ecologists should, therefore, measure a range of traits, establish plant trait redundancies and execute the statistical analyses. Research or dataset specific analyses would avoid the loss of information and secure the accurate calculation of multi-trait functional diversity indices (Botta-Dukát, 2005).

3.1.6. Functional Diversity Indices

Currently over 40 metrics of functional diversity have been proposed in the field of functional ecology. They can broadly be categorised according to the functional theory they underpin. The Biomass-Ratio Hypothesis places greater emphasis on the dominant species in a community, suggesting the composition of dominant trait values reflect the effects of community assembly rules and dictate the provisioning of ecosystem processes and services. This hypothesis subscribes to a unidimensional perspective and calculates community-weighted mean trait values (Grime, 1998).

The Functional Diversity Hypothesis was calculated using indices that relied on categorising plant species into discrete functional groups or types and functional diversity was represented as the number groups present (Ricotta, 2005). Functional groups and types were computed using hierarchical clustering approaches, which were biased according to the method dissimilarity metrics and/or linkage criteria employed. Research has indicated that functional diversity from clustering

approaches is much more sensitive to the choice of distance measures than the clustering algorithm (Poos, Walker and Jackson, 2009). Additionally, species' relative abundances have been ignored in defining functional diversity (Mason *et al.*, 2003). As a result, a set of desirable criteria and properties have been outlined, which concentrate on the use of quantitative traits to construct a multivariate trait space and the avoidance of using surrogate qualitative measures that introduce arbitrary and loss of valuable individual-level information, such that every species is viewed as a unique composite of traits that should be taken into consideration during calculations (Fontana, Petchey and Pomati, 2016). Accounting for species' relative abundances has also facilitated the decomposition of functional diversity into richness, evenness and divergence concepts together with elements of alpha, beta and gamma diversity (Mason *et al.*, 2005; Ricotta, 2005).

3.1.6a. Functional Richness

Functional richness has been defined mathematically as the minimum convex hull volume containing all species in a community, as shown in Figure-3.1.2. The convex hull is computed using the Quickhull algorithm (Villéger, Mason and Mouillot, 2008). Functional richness is thought to measure the occupancy of the niche space, and represents niche complementarity (Mason *et al.*, 2005). Mason *et al* (2013) found that Villéger's functional richness measures (FRic) have significant power in detecting niche complementarity when species composition is influenced by priority effects. This was further evident in changes in functional space dimensionality caused by community structure (Mouchet *et al.*, 2010). Niche complementarity, here, describes the intrinsic functional insurance that can buffer against environmental fluctuations, resist invasion or contribute to ecosystem functioning (Schleuter *et al.*, 2010).

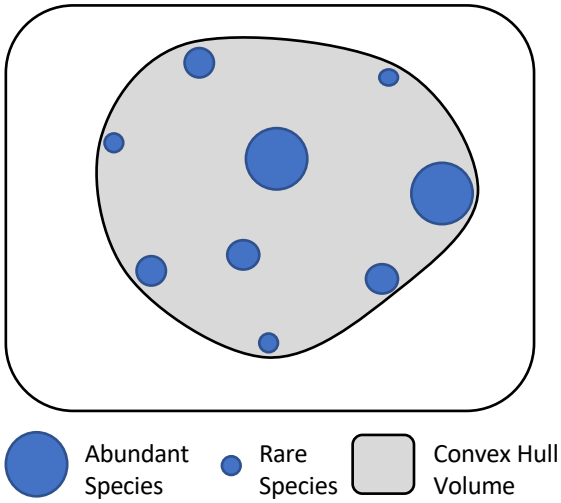

Figure-3.1.2. 2D representation of functional richness as defined by the total convex hull volume that species occupy in the trait space constructed by the most species.

3.1.6b. Functional Evenness

Functional evenness is a measure of regularity in the distribution of abundance in a dimensional space. Mouillot *et al* (2005) developed the functional regularity measure (FRO) for single dimensional trait spaces and Villéger, Mason and Mouillot (2008) presented a multidimensional measure. In essence, the regularity of the minimum sum of branch length is calculated from a minimum spanning tree together with the evenness in species abundance (Figure-3.1.3.).

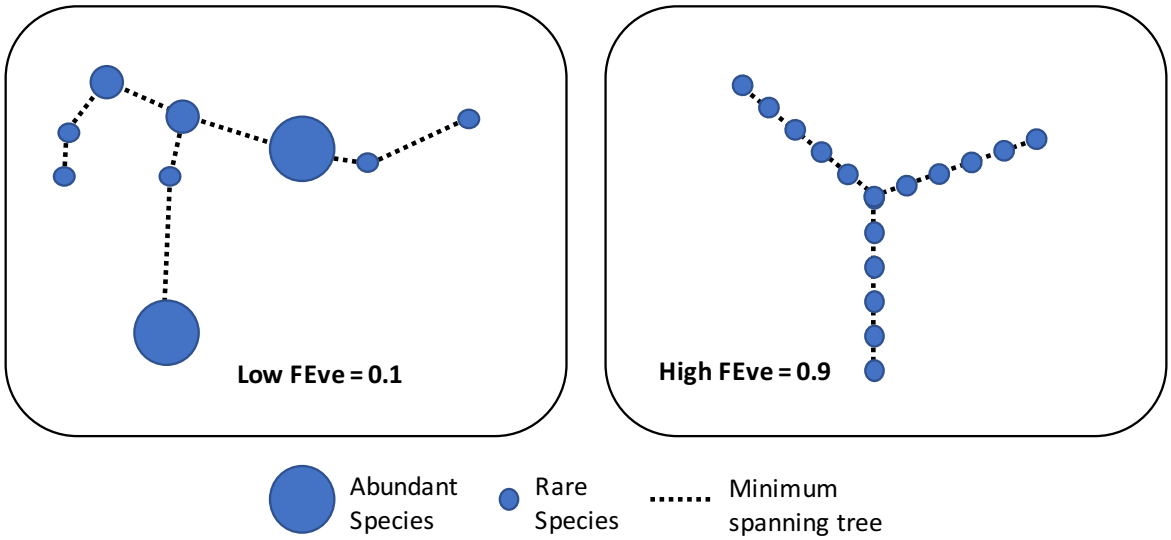

Figure-3.1.3. 2D representations of functional evenness as defined by the regularity of species distribution in the trait space weighted by their abundance.

Functional evenness is postulated to summarise the distribution of trait values in a dimensional space, and thus measures the utilisation of the trait space (Mouillot *et al.*, 2005; Villéger, Mason and Mouillot, 2008). It is assumed that greater utilisation of the trait space leads to greater efficiency of resources. FEve was found to reveal fine scale processes associated with competition for limiting resources and demonstrated the impacts of abiotic filtering (Biswas *et al.*, 2016).

3.1.6c. Functional Divergence

Functional divergence has been mathematically defined in various ways. The multi-trait indices can be segregated into two classes; one that relies on the species' distances from the centre of gravity (centroid) in the functional space, and the other the distances between individual species. The first class includes Villéger's functional divergence (FDiv) and Laliberté and Legendre's functional dispersion (FDis). FDiv quantifies how species (weighted by their abundances) are distributed within the volume of a multidimensional trait space with respect to the centre of gravity (centroid) in the functional space (Figure-3.1.4.). FDiv represents the sum of the deviances from the centroid, whereas FDis is the mean distance (Villéger, Mason and Mouillot, 2008; Laliberté and Legendre, 2010).

The second class includes Rao's Quadratic Entropy (Rao's Q) and mean nearest-neighbour distance (MNND). Rao's Q is derived from entropy theory and is expressed as the pairwise functional differences between species, weighted by their relative abundances, in a multidimensional space (Botta-Dukát, 2005). MNND is the mean distance to the nearest individuals in a multidimensional functional space, irrespective of species abundances, but can also be quantified for a unidimensional trait space. MNND and descriptive statistical measures (range, kurtosis, coefficient of variation, etc) are associated with single-trait indices of functional divergence.

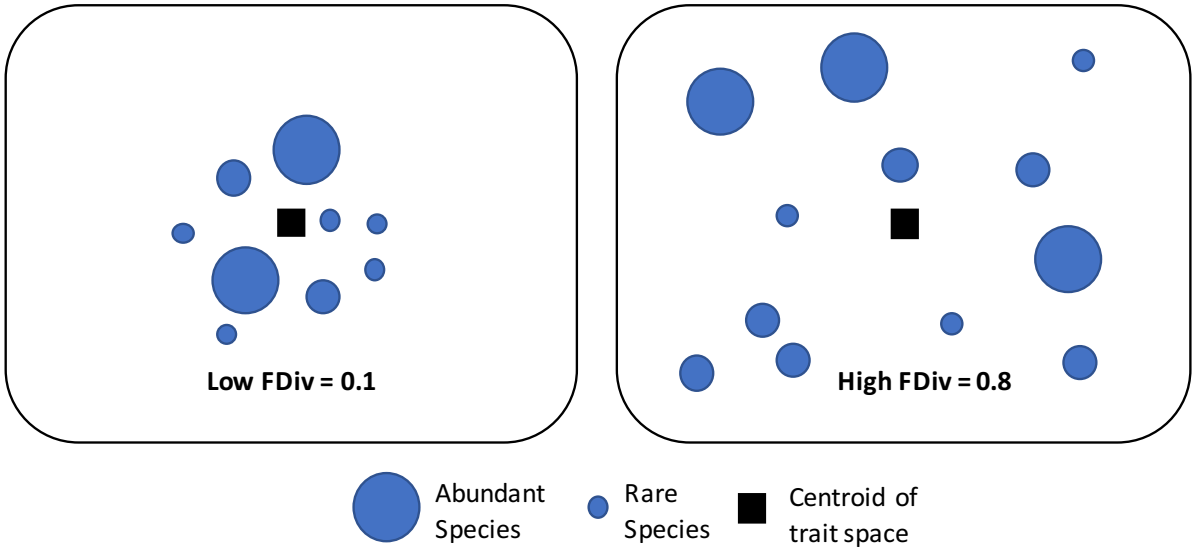

Figure-3.1.4. 2D representation of functional divergence as defined by the abundance-weighted distance of species from the centroid of the trait space.

All indices of functional divergence are suggested to reflect niche differentiation in resource usage to reduce competition (Mason *et al.*, 2005). The utility of Rao's Q has been realised in the decomposition of functional divergence into β and α elements, whereby βRao's Q represents the functional divergence among-communities, and αRao's Q the within-community functional divergence. αRao's Q is the traditional definition of Rao's Q. βRao's Q quantifies the degree of functional differentiation among biological communities at the local and regional scale, which can reveal community assembly mechanisms (de Bello *et al.*, 2010).

3.1.6d. The Performance and Redundancy of Functional Diversity Indices

Schleuter *et al* (2010) studied the performance of twelve functional diversity indices and found that all of them accurately measured what they were intended to describe. Despite this, a consensus on the performance of individual indices and their redundancy is lacking and ecologists are tasked with subjectively choosing robust and complementary functional diversity indices in practice (Mouchet *et al.*, 2010). Mouchet *et al* (2010) reaffirmed the segregation of richness, evenness and divergence indices and Villéger's indices (FRic, FEve and FDiv) were found to be statistically independent and were robust in measuring the functional responses to

community assembly rules, such as the effects of an abiotic filter (Mouchet *et al.*, 2010; Butterfield and Suding, 2013).

A key aspect in assessing the utility of functional diversity indices has centred on their redundancy, for example analyses have revealed Rao's Q is not independent from functional richness and divergence (Mouchet *et al.*, 2010). FDiv was reported to have outperformed Rao's Q and Mouchet *et al* (2010) echoed the speculation that FRic, FEve and FDiv are complementary and would enrich studies examining the responses and effects of biodiversity on ecosystem functioning. This has further been emphasised by Mason *et al* (2013) and Butterfield and Suding (2013). The latter authors suggested the implementation of single- and multi-trait indices in analyses of redundancy and ecosystem functioning. Multicollinearity among functional diversity indices is known to produce spurious results and the main effects on ecosystem processes and services cannot be segregated. A comprehensive redundancy analysis of both single- and multi-trait functional diversity indices is currently lacking in the field. The investigations, thus far, have focussed on the analysis of multi-trait indices originating from *in silico* communities (Villéger, Mason and Mouillot, 2008; Mason *et al.*, 2013).

3.1.7. Scope of this Chapter

The Leaf Economics Spectrum has received considerable empirical support from analyses of varying spatial scales, and, since Díaz's *Nature* publication in 2015, functional ecologists have focussed on their research on a reduced number of core axes of ecological specialisation, and critics have questioned their universality at different spatial scales. Additionally, redundancy analysis of univariate and multivariate functional diversity indices has been limited to artificial communities.

3.1.7a. Hypothesis 1: Ecological Axes of Specialisation of Temperate Grasslands

Global analyses have revealed the identity of three major axes: Leaf Economics Spectrum, Plant Height Axis and Seed Mass Axis. These three spectra will be consistent in the datasets (Park Grass Experiment and North Wyke Farm Platform) representative of temperate grasslands.

3.1.7b. Hypothesis 2: Redundancy of Functional Diversity Indices

Following the literature and redundancy analyses, the use of complementary designed indices (FRic, FEve and FDiv) is the most logical approach. These three indices will, therefore, be revealed in the analyses as being independent of each other, and dimensionality reduction will retain these indices.

The aim of this chapter is to reveal the underlying ecological axes of specialisation specific to temperate grasslands. Using the Park Grass Experiment and North Wyke Farm Platform datasets, comparisons with published literature and the major spectra in plant functional ecology can inform future analyses of temperate grasslands, in general, and the plant traits to be investigated throughout this thesis. Understanding the underlying trait redundancy in temperate grassland will counter multicollinearity issues in the statistical approaches and in the calculation of multivariate functional diversity indices. A further aim of this chapter is to investigate the complementarity of univariate and multivariate functional diversity indices to instruct the analyses in the succeeding chapters.

3.2. Methods

3.2.1. The Plant Traits

Decades of research have revealed a core list of ecological strategies and plant traits. The literature examined in this Chapter highlighted cornerstone plant traits of the Leaf Economics Spectrum, which are suggested to hold a high degree of redundancy, whilst the Plant Height and Seed Mass axes are postulated to be independent of this spectrum. These axes of specialisation have been associated with the ecosystem processes and services investigated in this thesis. The trait profile considered in this thesis, therefore, aimed to investigate plant traits of known importance in responses to environment and management factors (Chapter Four), and effects on biomass and food production (Chapter Five). Seven continuous traits were selected, and average trait values were requested and calculated from the TRY database (Kattge *et al.*, 2011).

3.2.1a. Plant Height: is defined as the shortest distance between the upper boundary of the main photosynthetic tissues, excluding inflorescences, on a plant and the ground level (Pérez-Harguindeguy *et al.*, 2013). Plant height is commonly linked to an individual's fitness and is suggested to be positively related to competitive vigour (Kraft *et al.*, 2015). Taller plants are said to be superior in pre-empting use of light resources via increased relative growth rate, whole-plant longevity and diaspore dispersal (Díaz *et al.*, 2015). This inevitably impacts community dynamics, which in turn, affects the provision of ecosystem processes and services. Taller plants are associated with greater biomass/fodder production (Lavorel and Grigulis, 2012). Additionally, plant height strategies have been linked to grazing responses – notably grazing avoidance of shorter plants (Díaz, Noy-Meir and Cabido, 2001).

3.2.1b. Leaf Dry Matter Content (LDMC): is the oven-dry mass (mg) of a leaf, divided by its water-saturated fresh mass (g) (Pérez-Harguindeguy *et al.*, 2013). LDMC has been hailed as a core functional trait with the capacity to elucidate the impacts of functional traits, at the species- and community-level, on a plethora of community and ecosystem properties and processes (Lavorel *et al.*, 2011; Gardarin

et al., 2014). LDMC is frequently used as a proxy for other functional traits and for the overall performance of an individual or species, via the definition of ecological axes of specialisation (Schellberg and Pontes, 2012). Higher and constant species-level values of LDMC have been linked to a conservative strategy of resource acquisition (Martin *et al.*, 2009). This manifests itself as a lower leaf tissue density, lower relative growth rate (leaf elongation rate and length of leaf growth), and increased plant longevity (particularly leaf life span) (Ansquer *et al.*, 2009a; M. Duru *et al.*, 2009; Suter and Edwards, 2013; da Silveira Pontes *et al.*, 2015). These scale-up to community-level processes of increased community stability, decreased peak herbage mass, decreased forage digestibility, and therefore community-level secondary production (Michel Duru *et al.*, 2009; Garnier and Navas, 2012; Májeková *et al.*, 2016).

3.2.1c. Leaf Nitrogen Content (LNC): is the total amount of nitrogen per unit of dry leaf mass (g) (Pérez-Harguindeguy *et al.*, 2013). LNC reveals the ecological strategy an individual or species exploits; those with high and constant LNC values employ a resource acquisitive strategy (Gubsch *et al.*, 2011; Schellberg and Pontes, 2012). Higher LNC recordings are closely related with increased mass-based photosynthetic rate through the association of LNC with proteins essential to photosynthesis, such as ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) (Suter and Edwards, 2013). Decreased leaf life span and leaf respiration rate are also related to higher LNC values (Garnier and Navas, 2012). LNC is thought to regulate the amount, palatability and digestibility of green biomass, litter quantities and litter decomposability that impacts the pools of soil carbon, therefore, carbon sequestration (Fortunel *et al.*, 2009; Lavorel and Grigulis, 2012; Díaz *et al.*, 2015).

3.2.1d. Leaf Carbon:Nitrogen Content Ratio (Leaf C:N): Higher leaf C:N is associated with resource conservative strategy and invests large amounts of resources in structural tissues, such as, root and shoot biomass (Ansquer *et al.*, 2009a). This, in turn, reduces green biomass production but reduces dry matter losses in brown biomass during storage (Byrt, Grof and Furbank, 2011). Leaf C:N negatively affects the palatability and digestibility of green biomass, and thus secondary production (Pérez-Harguindeguy *et al.*, 2013).

3.2.1e. Leaf Thickness: is related to the both SLA and LDMC: $(SLA \times LDMC)^{-1}$ (Laliberté *et al.*, 2012). Thicker leaves are commonly associated with resource conservative strategy (Lavorel and Grigulis, 2012). In particular, thicker leaves have lower mass-based photosynthetic rate due to slower carbon dioxide diffusion, and investment in the physical strength of foliar structures. Consequently, thicker leaves have a longer leaf lifespan (Pérez-Harguindeguy *et al.*, 2013). Altogether, thicker leaves are negatively associated with primary production and secondary production (palatability and digestibility).

3.2.1f. Specific Leaf Area (SLA): is the one-sided area of a fresh leaf, divided by its oven-dry mass (Pérez-Harguindeguy *et al.*, 2013). SLA is frequently associated with the resource acquisition strategy; individuals and/or species with high SLA values scale positively with relative growth rate, biomass production, secondary production (digestibility), leaf litter decomposition, and negatively to carbon dioxide capture and mass-based net photosynthetic capacity, and leaf lifespan (Cornwell and Ackerly, 2009; Suter and Edwards, 2013; Gardarin *et al.*, 2014). Laughlin (2014) suggested that SLA is critical to an individual's competitive vigour, particularly to maximise light interception with high SLA values. Establishment success has also been related to higher SLA values, whereas lower records are associated with individual persistence due to extra structural strength through the allocation of tannins, phenols and/or defensive compounds (Westoby, 1998; Marteinsdóttir and Eriksson, 2014).

3.2.1g. Seed Dry Mass: is the oven-dry mass of an average seed of a species (Pérez-Harguindeguy *et al.*, 2013). Seed mass is commonly linked to an individual's and/or species' community dynamics – dispersal, establishment and persistence (Garnier and Navas, 2012). Seed mass was found to be negatively correlated with seed output, dispersal distance, persistence in the seed bank, and positively with the relative growth rate and the establishment and persistence of seedlings (Carboni *et al.*, 2016). These positive associations are due to greater reproductive investment in individual seeds, which produces seeds of greater quality – larger reserves to facilitate respiration during periods of carbon deficit (Garnier and Navas,

2012; Fischer, Von der Lippe and Kowarik, 2013). Nishizawa & Aarssen (2014) found that seed mass was generally a good predictor of seed nitrogen content. Relationships between seed mass and ecosystem processes/services have been limited, but Roscher *et al* (2013) did demonstrate a positive relationship between community-level seed mass and community biomass.

3.2.2. Computation of Functional Diversity Indices

The choice was made to focus on univariate (community-weighted mean, functional regularity, mean nearest neighbour, range) and multivariate measures (FRic, FEve, FDiv, FDis, mean nearest neighbour distance, Rao's Q: alpha and beta) of functional diversity. These indices were computed using two matrices: Species X Plant Trait and Site/Plot X Species. The matrices were compiled according to the Park Grass Experiment and North Wyke Farm Platform. Functional diversity indices were computed in the R environment using the *FD* package (function: *dbFD*). Rao's Q was decomposed into alpha and beta elements using the *Rao* functional published by de Bello *et al* (2010).

3.2.3. Statistical Analyses

Principal component analyses, based on the correlation matrices, were conducted to examine the independence and redundancy of plant traits and functional diversity indices. Matrices of Species x Plant Traits and Site/Plot x Functional Diversity Indices were constructed, standardised and submitted for PCA. The number of dimensions to be retained was based on coordinates (<0.2) on the main PCA axes as determined by the number of components needed to explain total variance between 70-80%. Pairwise relationships between each individual trait and functional diversity indices were investigated using Spearman's coefficient of correlation.

3.3. Results

3.3.1. Ecological Axes of Specialisation of Temperate Grasslands

Spearman correlations between the plant traits revealed a weak correlation structure (Figure-3.3.1.). The highest significant correlation was found between plant height and leaf dry matter content (r = 0.44). The first four components of the PCA accounted for 77.73% of the total inertia (Table-3.1.1.). The absolute loading demonstrated that all seven traits should be retained for future analyses (eigenvectors >0.2). The findings contrast with previous research on ecological axes of specialisation and ecological strategies, as each of the seven plant traits were found to be independent and were used in the calculation of the univariate and multivariate functional diversity indices in 3.3.2.

	PCA1	**PCA2**	**PCA3**	**PCA4**
Eigenvalues	2.000	1.476	1.133	0.943
Variance Explained (%)	28.58	21.08	16.19	11.88
Cumulative Variance Explained (%)	25.58	49.66	65.85	77.73
Plant Height	0.015	-0.360	-0.534	0.314
Leaf Dry Matter Content	-0.371	-0.612	0.157	-0.274
Specific Leaf Area	0.559	-0.004	0.299	0.429
Seed Mass	0.086	-0.272	-0.611	0.146
Leaf Thickness	-0.231	0.645	-0.428	-0.106
Leaf Nitrogen Content	0.578	-0.037	-0.162	-0.141
Leaf C:N	-0.394	0.066	0.135	0.768

Table-3.3.1. Results of principal components analysis with eigenvectors for axes 1 to 4 between mean plant trait values acquired from the TRY database.

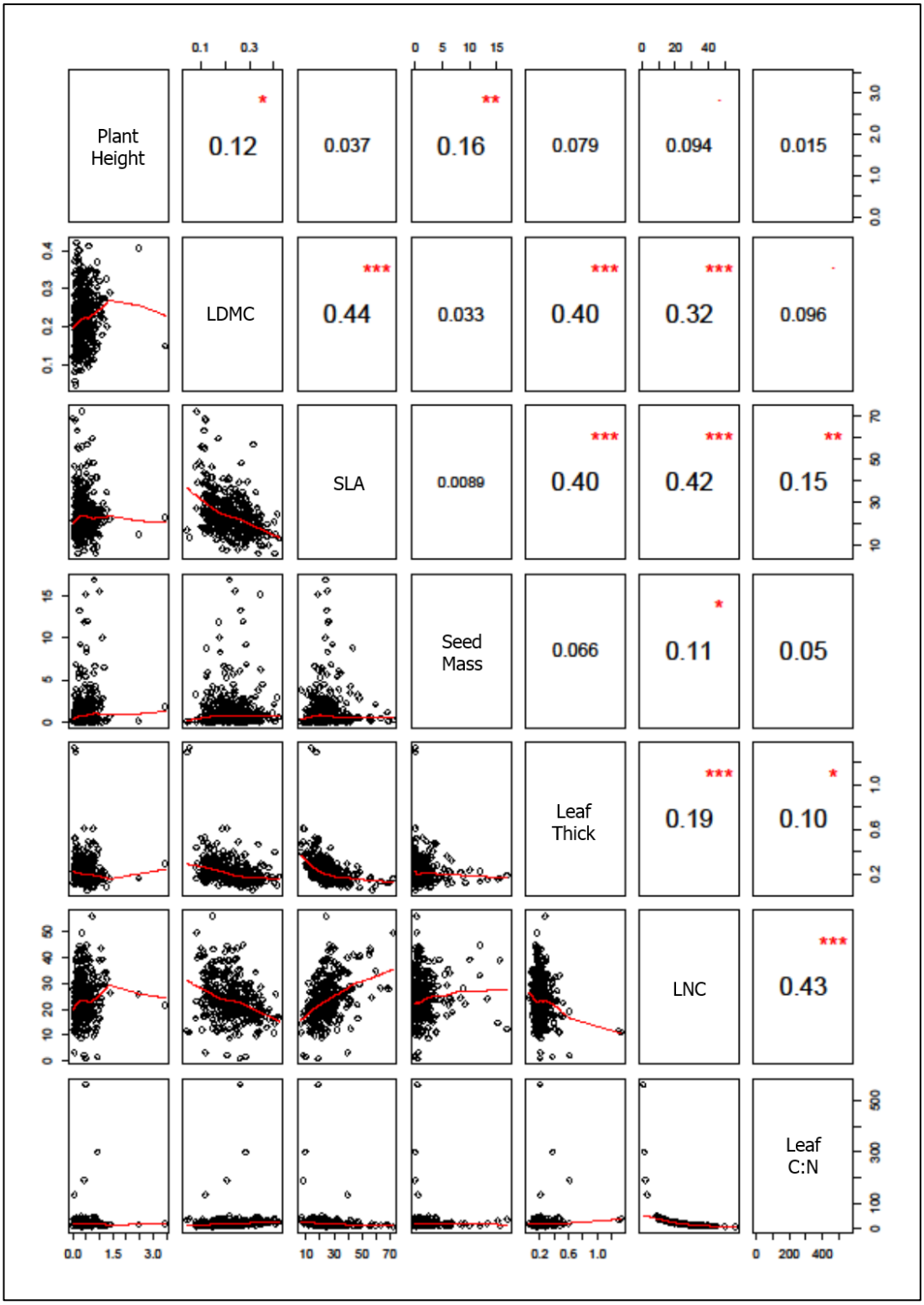

Figure-3.3.1. Associations among seven plant traits used in this project shown via bivariate plots (lower panels) and Pearson's correlation coefficients (upper panels) with the p-values (= p<0.05, ** = p<0.01, *** = p<0.001). Abbreiviations: LDMC, Leaf Dry Matter Content; SLA, Specific Leaf Area; LNC, Leaf Nitrogen Content.*

3.3.2. Redundancy on Functional Diversity Indices

The first five components were found to explain 78.47% of the total variance for the functional diversity indices. Of the thirty-five functional diversity indices, four univariate and two multivariate indices had absolute loadings of below >0.2 and thus were eliminated from future analyses. The loadings on the principal components highlight distinct segregations of the univariate and multivariate indices. The key results are demonstrated in the groupings of the multivariate functional diversity indices. Functional richness and mean nearest neighbour distance aligned with component one, βRao'sQ with component two, and functional evenness and divergence with component three. With regards to the partitioning of univariate functional diversity indices, there were clear affinities for the ranges of plant trait values with component one and functional regularity with component three. The loadings on the components highlight distinct groupings, for example principal component three encapsulates functional evenness at the single and multi-trait levels.

A		**PCA1**	**PCA2**	**PCA3**	**PCA4**	**PCA 5**
Eigenvalues		17.212	4.779	3.605	2.955	1.26
Variance Explained (%)		45.30	12.58	9.49	7.78	3.32
Cumulative Variance Explained (%)		45.30	57.88	67.37	75.15	78.47
Univariate Indices	CWM_{PH}	-0.044	-0.039	-0.145	0.448	0.274
	CWM_{LDMC}	0.020	0.179	-0.139	0.421	-0.213
	CWM_{SLA}	-0.044	0.199	-0.056	0.381	-0.134
	CWM_{Seed}	-0.044	-0.279	-0.099	0.169	0.454
	CWM_{Thick}	-0.026	-0.200	0.139	-0.466	0.156
	CWM_{LNC}	0.078	-0.350	-0.185	0.126	0.160
	$CWM_{C:N}$	-0.108	0.324	0.177	-0.072	-0.155
	$\boldsymbol{FRO_{PH}}$	-0.141	0.048	-0.024	-0.045	0.136

	FRO$_{LDMC}$	-0.167	0.066	-0.125	-0.043	-0.151
	FRO$_{SLA}$	-0.156	0.028	-0.234	-0.125	0.023
	FRO$_{Seed}$	-0.094	0.048	-0.354	-0.12	0.196
	FRO$_{Thick}$	-0.153	-0.005	-0.224	-0.145	-0.141
	FRO$_{LNC}$	-0.141	0.07	-0.284	-0.092	0.052
	FRO$_{C:N}$	-0.16	0.079	-0.234	-0.05	0.068

B		**PCA1**	**PCA2**	**PCA3**	**PCA4**	**PCA 5**
Univariate Indices	***MNND$_{PH}$***	0.087	-0.04	-0.156	-0.009	-0.196
	MNND$_{LDMC}$	-0.214	-0.088	0.005	0.023	-0.122
	MNND$_{SLA}$	0.204	-0.168	-0.015	0.067	-0.103
	MNND$_{Seed}$	0.169	-0.248	0.02	0.108	-0.092
	MNND$_{Thick}$	0.207	-0.182	-0.013	-0.004	-0.104
	MNND$_{LNC}$	0.157	-0.022	0.075	0.051	-0.072
	MNND$_{C:N}$	0.139	0.117	-0.227	-0.017	-0.255
	Range$_{PH}$	-0.201	-0.044	0.047	0.089	-0.054
	Range$_{LDMC}$	-0.217	-0.022	0.075	0.051	-0.072
	Range$_{SLA}$	-0.204	-0.104	0.038	0.169	0.013
	Range$_{Seed}$	-0.182	-0.13	0.11	0.129	-0.041
	Range$_{Thick}$	-0.205	-0.112	0.082	0.01	-0.091
	Range$_{LNC}$	-0.221	-0.05	0.096	0.024	-0.095
	Range$_{C:N}$	-0.219	0.01	0.117	-0.02	-0.115
Multivariate Indices	*FRic*	-0.223	-0.066	0.102	0.068	-0.018
	FEve	-0.157	0.045	-0.266	-0.087	-0.118

	FDiv	-0.175	0.06	-0.251	-0.101	0.061
	FDis	-0.192	-0.18	-0.02	0.098	-0.191
	αRao'sQ	-0.194	-0.184	-0.005	0.125	-0.096
	βRao'sQ	0.088	0.366	0.131	-0.022	0.195
	MNND	0.206	-0.068	-0.129	-0.028	-0.214

Table-3.3.2. Results of principal components analysis with eigenvectors for axes 1 to 5 between univariate and multivariate functional diversity indices calculated from the Park Grass Experiment and North Wyke Farm Platform. Indices in bold indicate those that had eigenvectors less 0.2 on every component.

3.4. Discussion

3.4.1. The Functional Dimensionality of Temperate Grasslands

The universality of defined ecological axes of specialisation has been challenged by these analyses. Research has indicated the significance and conservatism of the plant ecological strategies at varying spatial, geographical and ecological scales (Lavorel *et al.*, 2002; Díaz *et al.*, 2004; Freschet *et al.*, 2010; Pierce *et al.*, 2013). The reduction of plant trait dimensionality has been at the forefront of research to identify a core set of plant traits that encapsulate a species' ability to grow, survive and reproduce. The simplification of plant traits is thought to aid the application of dynamic global vegetation models that envelope community assembly rules and the scaling of ecosystem processes and services. This chapter aimed to investigate underlying axes of specialisation specific to temperate grasslands to advance the understanding of plant strategies across ecosystems and enrich the subsequent analyses in this thesis. It was hypothesised that evidence for the conservation of three spectra (Leaf Economics Spectrum, the Plant Axis and the Seed Mass Axis) would be found (Westoby, 1998).

Westoby's Leaf-Height-Seed Scheme (1998) assumed the independence of specific leaf area, plant height and seed mass. Pierce *et al* (2013) and Díaz *et al* (2015) condensed the latter two traits into a single Size Axis. This was suggested to represent a species' ability to cope with disturbances (disturbance adaptation strategy), and aligned with the C-R disturbance axis of Grime's CSR (Grime, 1998; Pierce *et al.*, 2013). Plant height and seed mass were expressed as dependents (positive correlates), whereby taller plants display greater maternal investment in seeds (greater seed mass) to ensure the survivability of seedlings in the face of pressures, such as carbon deficits and interspecific competition. The pursuit of a single axis to explain plant species' competitive ability has resulted in the incorporation of other size-related plant traits of above-ground organs, such as stem diameter and leaf area (Dirks *et al.*, 2017). A weak correlation ($r = 0.16$) was reported for plant height-seed mass in this chapter. This finding challenges the integration of multiple traits into a single axis and supports Westoby's original concept of plant height and seed mass as separate dimensions.

Westoby *et al* (2002) initiated the development of the Leaf Economics Spectrum and its associated exploitative-conservative ecological strategy. Decades of research have supported use of the Leaf Economics Spectrum across a broad range of geographic regions and biomes (Wright *et al.*, 2004; Freschet *et al.*, 2010). The coordination of morphological, biochemical and metabolic plant traits has been widely documented and was summarised in Table-3.1.1. The results of the current analysis, however, show independence between the plant traits previously thought to co-vary along the Leaf Economics Spectrum; the highest recorded Spearman correlation coefficient ($r = 0.44$) revealed a weak relationship between specific leaf area and leaf dry matter content. These traits together with leaf thickness and leaf nitrogen have been hailed as core components of the Leaf Economics Spectrum, and the exploration of a single representation of plant traits has focussed on the ratios of interspecific and intraspecific variation (Westoby *et al.*, 2002). This chapter postulates that this reductive paradigm fails to hold at the ecosystem-level (temperate grasslands), and there is potential for losses of valuable ecological information. Wright *et al* (2005) echoed this notion and suggested a multitude of different axes segregating leaf nutrient contents. Variation in leaf nutrient contents was previously assumed to be sufficiently captured by leaf nitrogen content. In fact, Wright *et al* (2005) found leaf nitrogen content to co-vary with phosphorus and was independent of two further axes; 1) leaf calcium, magnesium and potassium content, and 2) leaf manganese content. This suggests that reducing the Leaf Economics Spectrum down to leaf nitrogen content, as recommended by Lavorel and Grigulis (2012), would result in the loss of complex biochemical information together with metabolic and morphological aspects.

Future investigations should conduct a preliminary redundancy analyses of plant traits and define unique spectra. This approach would account for morphological, metabolic and biochemical leaf traits, but holistic analyses are significantly lacking in the literature and this chapter can be criticised on the same basis. The greatest hindrance to these investigations is the lack of metabolic trait data, for example photosynthetic rate per leaf dry mass is recorded for 1.63% of plant species in the TRY database (Kattge *et al.*, 2011).

3.4.2. Univariate and Multivariate Functional Diversity Indices

Choosing a complementary suite of functional diversity indices has relied on the opinions and experiences of functional ecologists (Mouchet *et al.*, 2010). The redundancy analyses conducted thus have focussed on the analysis of multivariate functional diversity indices with little consideration of univariate indices. These investigations calculated functional diversity indices from artificial data with the primary aim of examining the performance of functional diversity indices under different assembly rules (Butterfield and Suding, 2013). The present study aimed to investigate the complementarity of functional diversity indices and reduce multicollinearity among indices to avoid the production of false results and interpretations. Villéger, Mason and Mouillot (2008) defined and indexed three complementary facets of functional diversity; richness, evenness and divergence. The extent to which univariate and multivariate indices together provide unique information has been ignored in functional ecology (Butterfield and Suding, 2013). The principal component analysis based on the Park Grass Experiment and North Wyke Farm Platform data revealed that the univariate and multivariate functional diversity indices were scattered across five components. The indices detailed by Villéger, Mason and Mouillot aligned with components one and three. Redundancy was found in the functional divergence indices FDiv, FDis and αRao's Q.

Component one was characterised by the ranges of all seven plant traits and the multivariate index: functional richness. It is postulated that these indices align with the first component due their mathematical similarities. Functional richness measures the volume of the convex hull based on the most distal species weighted by relative abundances (Villéger, Mason and Mouillot, 2008). This measure thus accounts for the range of plant trait values in a multidimensional space and accounts for extreme values. Range, on the other hand, calculates the spread of trait values in a one-dimensional trait space. Functional richness quantifies the occupancy of the niche space and is parallel to niche complementarity, whereas range has been suggested to be a univariate measure of functional divergence and niche differentiation (Mason *et al.*, 2005; Aiba *et al.*, 2013). Regardless, both of these ecological concepts underpin the concept of functional insurance that secures species' longevity in the face of abiotic and biotic perturbations and supports the delivery of ecosystem processes (Schleuter *et al.*, 2010).

The third component was represented by univariate and multivariate measures of functional evenness. It is not surprising that these measures aligned with the same component as they capture the uniformity in the distribution of species abundances in a univariate or multivariate dimensional space (Mason *et al.*, 2005; Mouillot *et al.*, 2005). The extent to which functional regularity and evenness reveals unique information is unknown but what can be gleaned from the results is that the single- and multi-trait variates measure the same functional diversity component. Mason *et al* (2005) and Mouillot *et al* (2005) postulated this component reflected the utilisation of the trait space in that an equally exploited niche space elicits efficient resource use.

The principal components analysis conducted on five multivariate functional divergence measures resulted in the elimination of FDis and αRao's Q. Redundancy in Rao's Q was also reported by Mouchet *et al* (2010). Rao's Q has been suggested to embrace two of the functional diversity components of functional richness and divergence in that it is dependent on the range of the functional space occupied (functional richness) and on the similarity between dominant species (functional divergence) (Botta-Dukát, 2005). Furthermore, Fontana, Petchey and Pomati (2016) stated that functional dispersion and Rao's Q were not independent. This study decomposed Rao's Q into β (between communities) and α (within communities) because research has hailed the potential of Rao's Q to consistently detect community assembly rules under different trait scenarios (Mason *et al.*, 2013; Botta-Dukát and Czúcz, 2016; Fontana, Petchey and Pomati, 2016). The retainment of βRao's Q thus will enrich the analyses in future chapters. Between-community functional divergence has rarely been considered in functional trait analyses despite there being considerable utility in understanding the drivers of between-community variation. The employment of both within- and between-community indices is postulated to disentangle the effects of the abiotic and biotic filters – an aspect previous studies have encountered.

3.4.3. Future Directions

3.4.3a. Expansion of Plant Traits

Research in functional ecology, and in this study, can be scrutinised for disregarding plant root traits. Reich *et al* (2003) suggested that a single multiple trait axis does exist, and research has found strong association between above- and below-ground plant organs that align with the Plant Economics Spectrum. Grime *et al* (1997) found strong evidence of functional integration in resource competition dynamics between roots and shoots. Specific root length is thought to be the belowground variate of specific leaf area (Lavorel *et al.*, 2002). The intrinsic dimensionality of root traits has been suggested to be analogous to foliar patterning; low specific root length has been associated with higher longevity, root tissue density, low root respiration and high root C:N (Craine *et al.*, 2002). Lavorel *et al* (2002) added an increase in root diameter and branching density together with low nitrogen concentrations characteristic of slow growing species in nutrient-poor environments. However, Craine *et al* (2002) revealed that microbial symbioses with plant roots can alleviate the pressures of a nutrient-poor environment. Nitrogen-fixing bacteria were found to confer avoidance of nutrient limitation and the presentation of a trait profile characteristic of nutrient-rich environments (Craine *et al.*, 2002). This suggests that symbiotic relationships have the capacity to modify ecological axes of specialisation. Currently, a plant trait quantifying fungal and bacterial symbiotic relationships is lacking in the Thesaurus of Plant Characteristics but the Ecological Flora of the British Isles does document associated fungal relationships (Fitter and Peat, 1994; Garnier *et al.*, 2017). Configuring a plant trait encapsulating symbiotic relationships would enrich the study and definition of ecological axes of specialisation.

3.4.3b. Towards a Core List of Plant Traits

The ability of different spatial and/or ecological scales to mediate spectra was realised in the results of this chapter. Global axes of specialisation were found to be inconsistent at the ecosystem-level (temperate grasslands) and it has been reported that small-scale studies have failed to confirm the presence of the Leaf Economics Spectrum (Messier *et al.*, 2016). Messier *et al* (2016) further argued that broad ecological axes of specialisation may explain what plant trait profiles are locally

viable. This line of thought challenges the utility of global spectra and urges the partitioning of existing spectra, for example a comprehensive analysis of the Leaf Economics Spectrum and Size axis at the ecosystem, community, neighbourhood, population, module and metamer-levels. Further research would, therefore, sample a whole host of ecosystems at the site, plot, individual plant, and plant organ levels to identify the scales conducive to ecological spectra. It is likely that independence of plant traits is rife at smaller-scales, as trait variation may reflect the environmental and management drivers of individual traits. Thus, it is vital for functional ecologists to identify the trait dimensions expressed in their datasets rather than interpreting the patterning of a few plant traits as simplified ecological strategies, such as leaf economic traits, as resource exploitative or conservative.

3.4.3c. The Univariate and Multivariate Functional Diversity Indices

Guidelines on the usage of functional diversity indices have relied on the *in silico* modelling of community assembly processes and the influence of biodiversity on the provision of ecosystem processes. Rarely have investigations considered comprehensive *in situ* measurements of species abundances and trait values. Artificial investigations have indicated that FRic, FEve and FDiv are complementary and Rao's Q was found to be inferior to FDiv (Mouchet *et al.*, 2010; Butterfield and Suding, 2013; Mason *et al.*, 2013). Redundancy in Rao's and FDis was also reported in this chapter in the use of a quasi *in situ* dataset. Fontana, Petchey and Pomati (2016) conducted a comprehensive analysis of functional diversity indices using individual-level trait values and species abundances. They found that the multivariate indices (FRic, FEve and FDiv) were inaccurate and unreliable for studies integrating intraspecific variation and advocated the use of FDis. Additionally, they devised alternatives to FRic and FEve: Trait Onion Peeling and Trait Even Distribution. Trait Onion Peeling calculates functional richness through summing the areas of convex polygons as dictated by the outermost individuals (Figure-3.4.1.). This was suggested to resolve the criticism that the FRic disregards a large number of individuals by considering the outer most convex (Area 1 in Figure-3.4.1.) (Fontana, Petchey and Pomati, 2016).

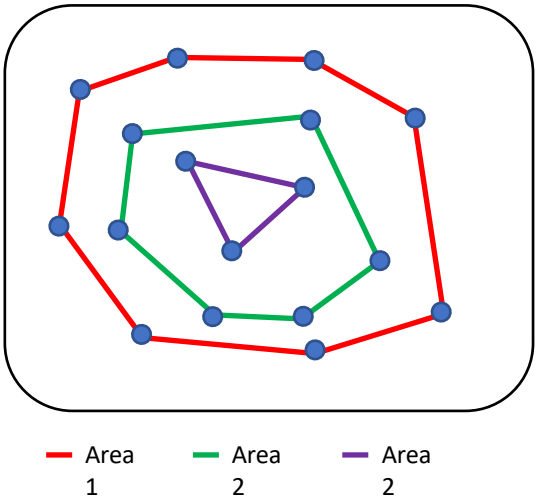

Figure-3.4.1. 2D representation of the Trait Onion Peeling index as proposed by Fontana, Petchey and Pomati (2016). Each blue point represents an individual organism and its position in the two-dimensional trait space. The perimeters of the three convex polygons are serially constructed and the areas measured and summed to calculate the Trait Onion Peeling index.

Examining the Functional Diversity and Convergence and Divergence Patterns in Response to Environmental Factors and Management of Temperate Grassland Communities

4.1. Introduction

The management of temperate grasslands is thought to have begun in the New Stone Age, circa 10000 BC, when large-scale deforestation started (Kaplan, Krumhardt and Zimmermann, 2009). Managed herbivory has been found near Neolithic communities through macro-remains analysis of the grazing-tolerant *Phleum pretense* (Hejcman *et al.*, 2013). Intensification of temperate grasslands in the 18th or 19th century produced pastures, meadows and grazed meadows; pastures are managed by livestock grazing, meadows are regularly cut, and grazed meadows are cut in spring then grazed in summer and/or autumn (aftermath grazing) (Pavlu *et al.*, 2007). Pastures are assumed to be low-productive systems, and meadows high, according to a plant species richness and composition perspective (Hejcman *et al.*, 2013). Intensification is the process of agricultural improvement, such as the addition of mineral fertilisers and tillage followed by reseeding with productive grass and legume varieties (Semelová *et al.*, 2008; Hejcman *et al.*, 2013). Agricultural improvement was performed to increase the yield of primary and secondary production, and thus economic yield (Bullock *et al.*, 2011). Improvement of temperate grasslands was found to boost financial output by 200-600% (Hodgson *et al.*, 2005).

The benefits of nitrogen, phosphorus and potassium fertilisation have been documented in increasing the quantity and quality of pasture production and livestock output (Hopkins and Wilkins, 2006). Mineral fertilisers became widely available in the 19th century, and their application rose between the late 1960's and early 1980's – nitrogen fertilisers increased by 7% each year and topdressing with basic slag and superphosphate raised the productivity of relatively infertile pastures by promoting the growth of *Trifolium repens* (Lazenby, 1981; Mark, 1993; Hopkins and Wilkins, 2006). Cultivars of highly productive grasses and legumes were sown

at the end of the 20th century, typically perennial ryegrass and clovers in seed mixtures of one or two species (Hopkins and Wilkins, 2006). Cultivated varieties and varietal improvement selected for domestication syndrome traits in harvesting and propagation to breed genotypes that were fast germinators, leafy and persistent with the intentions of increasing sward production and forage nutritional value (Hopkins and Wilkins, 2006; Schröder and Prasse, 2013). Cultivar sowing and subsequent fertilisation decreased floristic richness, which had knock-on negative effects on invertebrate numbers and overall diversity (Pavlů *et al.*, 2012). Bullock et al (2011) suggested that agricultural grassland improvement will play a minor role in the future (2050) of temperate grasslands in the United Kingdom.

The greatest threat to present and future temperate grasslands has been identified as inadequate management, such as under-grazing, leading to rank vegetation, novel ecosystems, and/or forestation (Bullock *et al.*, 2011). It is suggested that 79% of temperate grasslands in the United Kingdom are in an unfavourable condition due to poor management because of poor financial returns from grazing and the lack of funding for conservation grazing (low intensity) (Bullock *et al.*, 2011). The extensification of temperate grassland management has been proposed as an alternative to intensification to increase the provision of multiple ecosystem services with diminishing stocking numbers and fertiliser inputs. This is to satisfy environmental objectives, such as reducing eutrophication by nitrate and atrazine, encourage fauna and flora conservation, water catchment, soil erosion control, and carbon sequestration. Extensification practices focus on better utilisation and quality of roughage and products by sowing perennial ryegrass and clovers (white clovers in grazed grasslands; red clover in temporary grasslands), optimum use of animal manure, and innovative solutions to reduce overall production costs (seeds purchase, soil tillage and nitrogen fertilisation) (Hopkins and Wilkins, 2006). However, extensive systems are suggested to be unsustainable and unreliable for farmers as sowing clovers are more expensive, communities are susceptible to random collapse, and yields are unpredictable and slower compared to perennial grasses and nitrogen fertilisation. In temperate regions, extensification is only feasible if there is a reduction in land taxes, income taxes levied, and farmers are educated to reduce variable (e.g. fertiliser) and fixed costs per hectare (e.g. buildings and machinery).

Research in the trade-off of ecosystem services between intensification and extensification of temperate grassland management is still embryonic. Literature has focussed on the provision of a single ecosystem service: plant diversity or food production. Plant diversity is assumed to quantify the intrinsic resilience and functioning of the ecosystem; higher diversity results in increased functionality – the Biodiversity-Ecosystem Function Hypothesis. The different facets of biodiversity have been associated with multifunctionality, such as taxonomic and genetic, but the leading component is functional diversity underpinned by plant functional traits.

4.1.1. Plant Functional Traits and Environmental/Management Pressures

The relationship of plant functional trait diversity and syndromes, and environmental and management pressures was explored using the abiotic filters proposed by Lavorel and Garnier (2002) as part of their Response-Effect Framework. They assumed that these abiotic filters constrain the range of response trait values that coincide with the fitness optima of the local community, causing adaptation in growth, survival, and reproduction. These response traits represent an individuals' or species' position within a multidimensional trait space - functional or fundamental niche (Violle and Jiang, 2009). The fundamental niche is merely a hypothetical space constrained by the environmental and management pressures; this translates into the realised niche when community dynamics (interspecific competition and facilitation) are considered and species abundances quantified (Schellberg and Pontes, 2012). Fundamental niches and realised niches are also termed pre-competitive and post-competitive niches. Lavorel and Garnier (2002) referred to this community dynamics' barrier as the biotic filter.

Whereas the abiotic filter is considered to be a convergent force, the biotic filter is suggested to cause divergence in response traits. The 'limiting similarity' hypothesis assumes that within a finite niche space the most competitive species occupy much of the niche space, and subordinate species differentiate to co-exist (Funk *et al.*, 2008). Competitive hierarchies have been constructed according to functional traits, predominantly plant height, specific leaf area and leaf nitrogen content, that closely

associate with resource capture and use and the interception of solar radiation (Laughlin, 2014b). Plant height is the most studied functional trait in terms of competition and has been strongly matched with species' competition coefficients (Park, Benjamin and Watkinson, 2003).

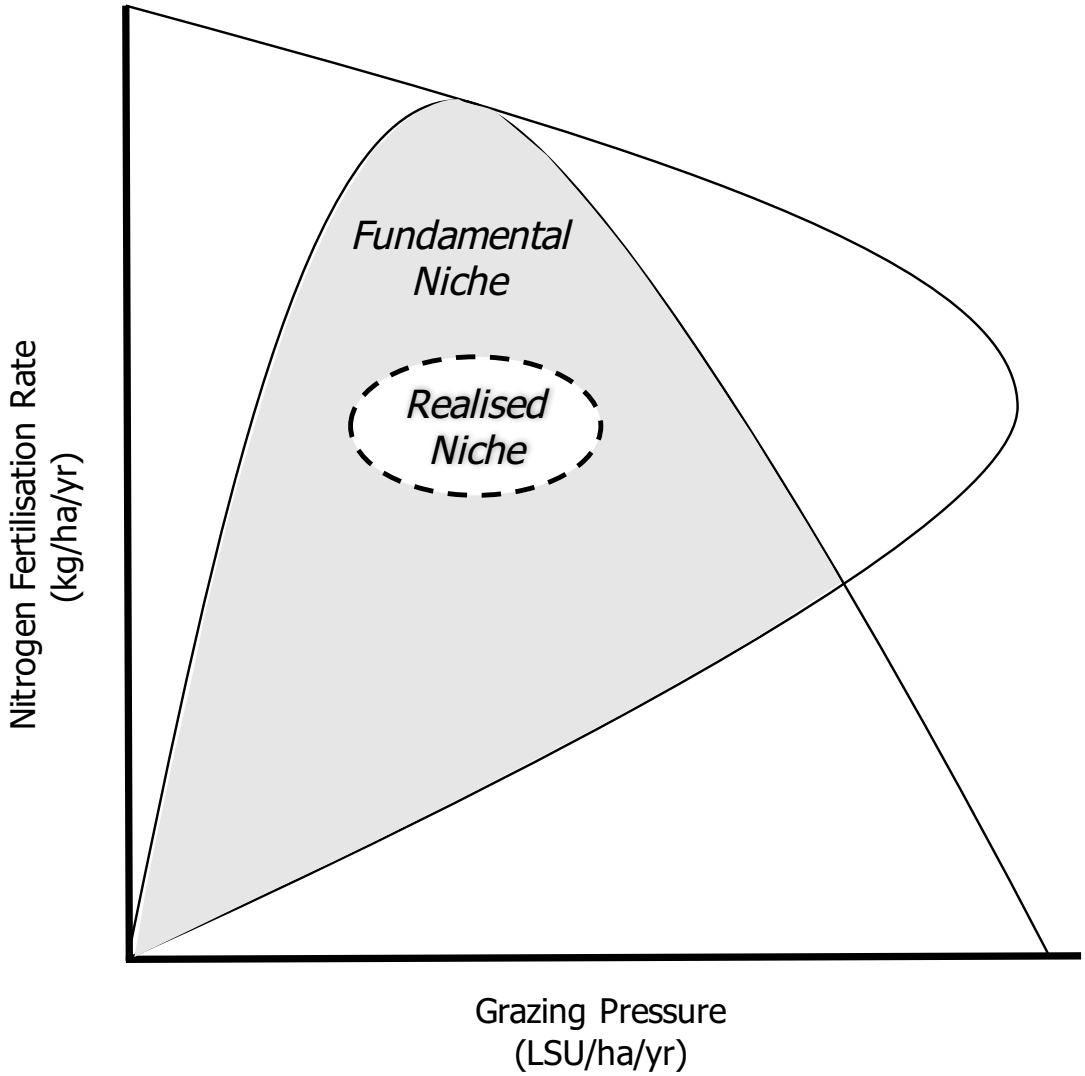

Figure-4.1.1. Graphical representation of the fundamental and realised niche concepts. The distribution curves represent fitness (as species abundance or community-weighted means of traits values) along the environmental and/or managements continuums or abiotic filters. The grey region refers to the hypothetical fundamental niche (habitable zone), which translates into the realised niche once the biotic filter (- -) has acted. The realised niche manifests post-community dynamics (interspecific competition and/or facilitation).

Facilitation is suggested to cause divergence by alleviating the impact of abiotic filters and thus widening the fundamental and realised niches. Examples within the literature focus on severe environments, such as deserts and alpine tundra systems but facilitation has been investigated in grasslands as an ecological restoration technique using nurse plants to improve water potential and the establishment of shrubs (Maestre *et al.*, 2001). However, the impacts of facilitation on plant functional traits along environmental gradients is under-studied and the net effects of facilitation and competition are rarely disentangled (Brooker *et al.*, 2008).

Responses of plant functional traits to environmental and management factors have typically been studied with regards to plant species' strategies; "exploitative vs conservative" and "tolerant vs avoidant"; and through the creation of indices that elicit a community's functional structure and composition. The simplest measure of community-level functional composition is the community-weighted mean trait value that is suggested to vary along environmental gradients (Ackerly and Cornwell, 2007). A substantial proportion of the literature has implemented this index in community responses, however, scaling issues at different spatial levels have been reported particularly from the species- to the community-level. Instead, further facets of functional structure and composition have been realised, specifically capturing the distribution of trait values in a community through functional diversity indices (Mason *et al.*, 2005). These indices are typically associated with the convergence-divergence paradox that aligns with the notion of abiotic filters selecting for a limited range of trait values. Three individual elements of functional diversity are thought to capture and quantify this paradox and quantify the intrinsic functional redundancy in a system. Functional richness represents the occupancy of the niche space and reflects the intensity of abiotic filters (Villéger, Mason and Mouillot, 2008). Functional evenness represents the distribution of the community within the niche space and is indicative of niche partitioning or differentiation that is associated with the divergent biotic filter (Cornwell and Ackerly, 2009). Finally, functional divergence infers whether abiotic or biotic filtering is operating strongly in communities (Villéger, Mason and Mouillot, 2008). Functional redundancy is an amalgamation of the three concepts that supposes a larger and more evenly occupied niche space guarantees continued provision of ecosystem processes (Rosenfeld, 2002).

The indices of functional structure and composition are rarely studied in conjugation. Single-trait indices summarise the complexity of responses to environmental gradients, and multi-trait indices demonstrate the nature of, and responses to, the multidimensional niche. Funk *et al* (2016) stressed the need to incorporate an array of indices to examine communities' responses to environmental and management factors. The coming sections concentrate on defining the key abiotic and biotic filters that underpin the key ecological strategies, which affect the functional structure and composition of temperate grasslands.

4.1.2. Plant Species' Strategies Along Nutrient Gradients

Historically, the edaphic environment of temperate grasslands has been intensively managed and researched with a particular focus on nutrients, predominantly nitrogen, phosphorus, and potassium, and their impacts on productivity and community assembly dynamics (Hejcman *et al.*, 2013). Soil nutrient status has been identified as a crucial abiotic filter, which impacts the position, availability, evenness, and breadth of the ecological niche space (Harpole and Tilman, 2007). A global analysis has further identified nutrient filters as a leading convergent force across all plant lineages, suggesting phylogenetic conservatism in species' responses to nutrient filters (Díaz *et al.*, 2004).

The optimal positioning in the niche space has typically been investigated through community-weighted mean traits along nutrient gradients and is suggested to provide a good assessment of the trait-environment relationship that elicits plant strategies. The leaf economics spectrum has been defined as a universal strategy that relates trait-environment associations with nutrient acquisition and use (Wright *et al.*, 2004). It characterises contrasting plant types, exploitative or conservative, based on their ability to colonise, grow and reproduce. Its core list of plant traits is leaf nitrogen content, specific leaf area and leaf photosynthetic rate, which have been highlighted as response traits to nitrogen supply (Schellberg and Pontes, 2012).

Maire *et al* (2009) highlighted the trait profiles of N-exploitative and N-conservative plant types in a study of thirteen perennial grasses of low-mountain grasslands. N-exploitative species were found to have high leaf nitrogen content, high root uptake capacity, and low leaf N use efficiency; N-conservative species exhibited the opposite profile. The co-variation of these plant traits was previously confirmed in the literature; leaf nitrogen content is positively related to root uptake capacity and negatively with leaf N use efficiency (Tjoelker *et al.*, 2005). This co-variation highlights that an N-exploitative type would display reduced above-ground productivity because of the decrease in leaf N use efficiency, thus N yield, and has been associated with higher relative growth rates and decreased leaf dry matter content (Schellberg and Pontes, 2012).

Functional responses in the Park Grass Experiment have been confined to the use of discrete taxonomic components (grasses, legumes, miscellaneous) (Silvertown, 1980). The balance of these taxonomic groups was suggested to be dictated by the availability of their own limiting resource; nitrogen in the case of grass species and minerals in the case of legumes. Species richness was found to decline from the control plots with the application of triple superphosphate alone, sodium nitrate or ammonium sulphate alone, nitrogen-based fertiliser and potassium sulphate together, farmyard manure, and triple superphosphate and potassium sulphate together (Crawley *et al.*, 2005). The application of ammonium sulphate saw the greatest reduction in species richness due to the replacement of short herb species by tall grasses, because of competition for light resources and the negative impacts of shading (Tilman and Isbell, 2015). The application of ammonium sulphate fertilisers exacerbates the strength of the abiotic filter, by decreasing the pH of the soil, which is suggested to lower the mineralisable nitrogen and organic matter through the absence of nitrifying bacteria and worms (Richardson, 1938). The application of lime counteracts this acidification and has been shown to increase species richness on plots receiving ammonium sulphate. Crawley et al (2005) concluded that on average, adding triple superphosphate loses six species; nitrogen-based fertilisation at 50kg/ha loses two species, with ammonium sulphate losing three more species than sodium nitrate; two extra species are gained for every unit increase in soil pH; using organic manures adds two species.

Trait response studies have largely focussed on nitrogen gradients with the assumption that all soils co-vary along this gradient. However, the type and nature of the nutrient has been suggested to be critical for species acquisition and use strategies, for example, grass species have been found to demonstrate preferences for the root uptake capacities between nitrate and ammonium to facilitate co-existence; dominant species preferring nitrate and rarer species ammonium (da Silveira Pontes *et al.*, 2015). Further research is needed to define plant responses and strategies for other soil nutrients, for example, phosphorus and potassium. The Park Grass Experiment offers a unique opportunity to investigate the plant trait responses to long-standing regimes of fertiliser and lime application.

4.1.3. Plant Species' Strategies of Grazing Tolerance and Avoidance

Grazing is one the main drivers of change in structure, composition, and functioning of grassland plant communities and the responses of plant species to defoliation have been linked to those common in high nutrient environments (Garnier *et al.*, 2007). High relative growth rates, associated with increased specific leaf area, have been considered as a key mechanism to tolerate defoliation. Gross et al (2007) demonstrated that the rapid growth rate of *Dactylis glomerata* compensated for the effects of disturbance. Grazing tolerance involves fast tissue regrowth (high specific leaf area) with very low structural defence (low dry matter content) and leaves that are palatable (high leaf nitrogen content) (Kahmen and Poschlod, 2004). Grazing tolerance is typically characteristic of adaptations to high nutrient environments, whereas, avoidance is linked to strategies in low productivity habitats (Cingolani, Posse and Collantes, 2005). Grazing avoidance strategies are associated with palatability, specifically the chemical composition of slow-growing plant organs with structural defences, such as high lignin content, low shoot cellulose, and low leaf nitrogen content. Avoidance is also typified by architectural and phenological changes to evade defoliation, for example, smaller plant stature and dormancy (Lloyd *et al.*, 2010). These two strategies are not mutually exclusive, as shown by Pontes et al (2010) who found a decrease in the number of leaves (grazing tolerance) and plant height (avoidance) simultaneously.

Research is continually searching for a plethora of responses of plant communities to defoliation. The work of Díaz *et al* (2001) demonstrated that prediction of grazing responses was best achieved when combining plant height, life history traits (annuals vs perennials) and leaf size. Size traits (plant height and leaf size) were again shown to be a component of grazing avoidance strategies. In Díaz's later research, climate and grazing history (intensity and frequency) were emphasised (Díaz, Sandra Lavorel, *et al.*, 2007). Further, the species of grazer has important consequences on the trait mosaic of a plant community, for example, cattle are more selective than sheep or horses and thus create uneven plant trait distributions, especially for leaf nitrogen content (Bartolome *et al.*, 2004). Targeted low-intensity grazing (stocking rates, timing, and species of grazer) has been used as a restoration technique to manage invasive plants in Europe, Australasia and the western United States. Rotational grazing of sheep was found to significantly reduce *Persicaria perfoliata* within a month and with synchronised efforts population growth rate was reduced through defoliation in the flowering season, thus reducing seed output, leading to an increase in native vascular plant species (Girard-Cartier and Kleppel, 2015).

The reintroduction of low-intensity grazing is realised to create a mosaic of suitable habitats to induce heterogeneity in temperate grasslands. Herbivory has the capacity to introduce spatial variability, thus creating new niche spaces. Defoliation alleviates competition for light interception (Muller *et al.*, 1998). Trampling introduces microsite creation that can potentially differ in soil moisture status, and carbon and nitrogen pools, which can also be influenced by the deposition of urine and faeces – the creation of new germination and establishment niches (Doll *et al.*, 2011). The establishment of new species is suggested to cause plant trait divergence (De Bello *et al.*, 2013). Research on low-intensity grazing has a long tradition in Great Britain, but the ecological advantages are being realised in the European Continent due to an association of low-intensity livestock systems with the high nature conservation value of grasslands across Europe (Bignal and Mccracken, 1996; Rosenthal, Schrautzer and Eichberg, 2012).

Trait response studies of herbivory have generalised their conclusions at extreme spatial scales (local or global) and intensities (conservative or intensive) (Díaz, Noy-Meir and Cabido, 2001; Díaz, Sandra Lavorel, *et al.*, 2007). Further research is needed to define the impacts of herbivory at intermediate scales (regional) with varying intensities. For temperate grasslands, the impact of differing grazing regimes on regional functional structure and composition is of particular interest for managers and conservation programmes. The National Vegetation Classification offers an exceptional opportunity to study the impact of environmental and management pressures on temperate grasslands at the regional and national scales through characterising the functional structure and composition of low intensity (pastures), aftermath grazing (meadows) and intensively grazed (grazed meadows) temperate grasslands.

4.1.4. Plant Species' Strategies to Shading

Competition for light resources is a result of changing light quality, such as the intensity of red to far-red light intensity that impacts growth performance. Two plant strategies, shade avoidance, and tolerance have been documented to understanding community competition dynamics. Shade avoidance is centred on the perception of a low ratio of red to far-red wavelengths that results in adaptations in morphological traits, for example, leaf positioning in higher strata accelerated flowering and elongation of hypocotyls, and apical dominance (Franklin, 2008). Consequently, carbon investment into above-ground architecture comes at the expense of root and leaf development, thus reducing the quantity and quality of these organs. The variations observed in plant height elicit complementarity for light resource and could suggest saturation of the ecological niche space. Shade avoidance is commonly seen in grassland systems (Gommers *et al.*, 2013).

Shade tolerance, on the other hand, is more complex; two contrasting hypotheses have been proposed. The "carbon gain hypothesis" defines shade tolerance as the maximisation of light capture in low light environments, whereas, the "stress tolerance hypothesis" focusses on the resistance to biotic and abiotic stresses in the understory (Givnish, 1988; Kitajima, 1994). These hypotheses are not independent, and the suite of traits associated with shade tolerance are applicable to both. Shade

tolerators, generally, allocate metabolic energy to enhance leaf survival at the expense of leaf elongation and optimal photosynthesis; increases in leaf dry matter content with decreases in specific leaf area and leaf nitrogen content (Valladares and Niinemets, 2008). The investment in leaf dry matter content increases leaf lifespan ("carbon gain hypothesis"), together with reducing palatability for herbivores ("stress tolerance hypothesis"). The shade tolerance strategy, therefore, has a very similar trait profile to that of nutrient conservatives.

Avoidance and tolerance can be extended to the concepts of dominants and subordinates respectively. The underpinning assumption is that plant traits predispose individuals/species to be dominants or subordinates with regards to competition for light resources – divergence is thus portrayed. However, at what spatial scale competition is evident or even relevant is still contested. Research employing the same functional structure and composition indices at different scales is thought to shed light on this dispute.

4.1.5. Scope of the Chapter

Finer clarification of the effects of environmental and management pressures on the convergence-divergence paradox is needed, but it is generally accepted that the abiotic filter induces convergence at the single- and multi-trait level. A general hypothesis for this chapter aligned with this notion of convergence. This chapter further proposed and examined three specific hypotheses to reveal the finer details of convergence and divergence patterns.

4.1.5a. Hypothesis 1: Grazing Regimes of Temperate Grasslands

Trait responses to grazing have come to fruition through the conceptualisation of tolerant and avoidant individuals/species influenced by decades of research at fine spatial scales. Díaz *et al* (2001, 2007) examined the impacts of herbivory on plant traits at the global scale and recognised the need to diversify research with regards to grazing intensities. Controlled grazing of temperate grasslands has produced pastures, meadows and grazed meadow, which would benefit from a collective plant trait-based analysis. The plant traits significantly impacted by grazing intensity

will be associated with those that align with the grazing tolerance strategy, moreover, increased grazing intensity will cause convergence in these plant traits.

4.1.5b. Hypothesis 2: Improvement Status of Temperate Grasslands

The conservative-exploitative continuum defined individuals/species with regards to their response trait values, thought to represent fitness optima along environmental gradients. The agricultural improvement of temperate grasslands, particularly through inorganic nitrogen fertilisation, has been widely recognised as a major driver of change in temperate grasslands since the 1940's to the present day (Bullock *et al.*, 2011). The effect of improvement status on temperate grasslands at a broader spatial scale is unclear, as well as the impact of farmyard manuring. Improved temperate grasslands will exhibit a trait profile and convergence/divergence patterns typical of exploitative individuals/species irrespective of organic or inorganic improvement.

4.1.5c. Hypothesis 3: Fertiliser Variety and the Functional Structure and Composition of Temperate Grasslands

The application of nitrogen-based fertilisers has shown patterns of convergence towards the plant traits characteristic of N-exploitative individuals/species, which often dominate improved temperate grasslands. Other fertiliser varieties are surmised to adhere to the same principle, but this has not been tested rigorously. Mineral fertilisation will, therefore, have similar influences on nitrogen-based fertilisation.

The aim of this chapter is to shed light on the convergence-divergence paradox in relation to environmental and management factors. Using the National Vegetation Classification and the Park Grass Experiment, analysing the impacts of previously under-studied factors (grazing intensities and mineral fertilisers) will increase the understanding of temperate grassland community structure and community and inform the management of the systems from a plant trait-based perspective. Increased knowledge allows ecologists and land managers to make appropriate

decisions to achieve conservation targets, such as the restoration or maintenance of multifunctionality through biodiversity.

4.2. Statistical Methods

4.2.1. Patterns of Trait Convergence and Divergence in NVC Communities/Sub-Communities

Patterns in plant trait convergence and divergence of the seventy-four (each with 500 replications) NVC communities and sub-communities was investigated using species occurrence data and through the calculation of fifty-five functional diversity indices covering components of richness, evenness and convergence/divergence at the single- and multi-trait level. All communities and sub-communities were categorised according to management regimes: all communities were divided according to grazing status (grazed, low intensity grazing and aftermath grazing), mesotrophic grassland (MG) according to improvement status and application of farmyard manure.

Using the community/sub-community presence/absence matrices and species traits matrices, fifty-five functional diversity indices were computed using the *FD* package (function *dbFD*)., together with published R codes for those not included in the *FD* package (range, mean nearest neighbour distance and functional regularity). Rao's Q was computed using R code published by de Bello et al (2010). A series of one-way analysis of variances (ANOVA) were conducted to examine the effect of management regimes on single and multi-trait functional diversity. Tukey's Honest Significant Difference (HSD) was used to discriminate significant differences between the means of management groups at the $p \leq 0.05$ significance level. Diagnostic plots were checked for heteroscedasticity and normality and those indices failing were log transformed.

4.2.2. Plant Traits-Environment Relationships in the PGE (1991-2000)

A combined approach of RLQ analysis and fourth-corner analysis, proposed by Dray et al (2014), was employed to tackle the problem of the fourth-corner, relating species traits to environmental variables (Figure-4.2.1.). RLQ is a multivariate ordination technique that aims to identify and summarise the main relationships between environmental gradients and plant traits mediated by species abundances, while the fourth-corner analysis evaluates the significance of bivariate associations

(each trait to each environmental variable) through correlation tests computed for each pair of variables. Separately, RLQ has been criticised for lacking significance tests and the fourth-corner corner-analysis for co-variation among traits or environmental variables. Therefore, a combined approach that applies fourth-corner tests on the ordination outputs of RLQ resolves these issues, and was the statistical method employed here to examine the relationships between plant traits and environmental variable in the PGE.

RLQ analyses were performed on the botanical survey dataset (PARKCOMPIC) conducted between 1991 and 2000. Three tables (R, L, and Q) were constructed. The L-tables (site-species abundances) were formulated, as shown in Figure-4.2.1., where each years L-table were combined to reveal general patterns in the Park Grass Experiment. The L-table was analysed using Correspondence Analysis (CA); the CA site scores were used as row weightings for a Hill-Smith Principal Component Analysis (PCA) of the R-tables (environmental descriptors for each site). The environmental variables were related to the fertiliser and liming regimes of the PGE, together with measures of grass species coverage, richness, diversity, and evenness. These biotic variables varied from year to year, whereas the abiotic descriptors were fixed. The Q-trait table was created from the trait data acquired from the TRY database. The Q-table was analysed by PCA, using CA species scores as column weightings. Subsequent RLQ analyses were conducted to combine the R, L, Q tables in a simultaneous ordination. To select the appropriate number of axes for each ordination, scree plots were examined and the number of components with eigenvalues greater than one were retained, except in the ordination of plant traits where seven axes were maintained in light of Chapter 3's results.

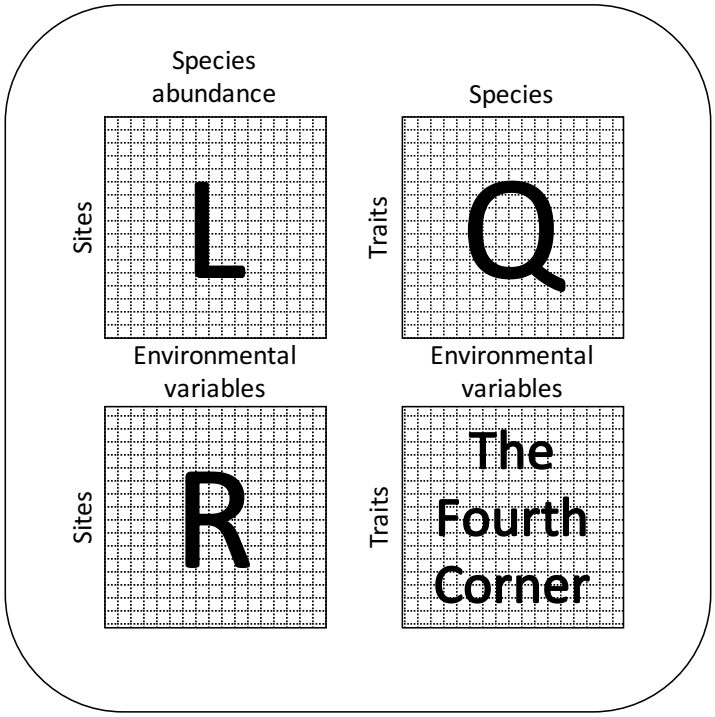

Figure-4.2.1. Representation of the fourth corner problem. Ecologists often generate tables L, Q, and R and employ an RLQ analysis to formulate the fourth corner matrix.

A multivariate test was applied to evaluate the global significance of the RLQ axes; Monte Carlo tests (3500 permutations) of two permutation models. Models 2 and 4 were employed to control type one errors (false positives) and combined (Model 6) to test the null hypothesis that "species are distributed irrespective of their traits and/or environmental conditions".

- Model 2: permutes the *n* samples (rows of table R- or L-table) to test the null hypothesis that the distribution of species with fixed traits is not influenced by the environmental descriptors.
- Model 4: permutes the *p* species (rows of Q- or columns of L-table) to test the null hypothesis that the species composition of samples with fixed environmental conditions is not influenced by species traits.
- Model 6: combines models 2 and 4 to test the null hypothesis that at least one table (R or Q) is not linked to L.

Statistical significance was found when the largest p-value, from Models 2 and 4, was less than or equal to 0.05. To evaluate statistical significance between RLQ axes and traits or environmental variables, fourth-corner tests, together with permutation Models 2 and 4, were computed. To reduce the possibility of false significance levels, the Benjamin-Hochberg correction was applied to recalculate significance levels for each relationship.

The combined approach of RLQ and fourth-corner analysis was conducted in the R environment using the *ade* package (functions: *dudi.coa, dudi.hillsmith, dudi.pca, rlq, randtest* and *fourthcorner.rlq*).

4.2.3. Using Environmental Variables to Explain the Functional Structure and Composition of the PGE

Using backward and forward stepwise multiple linear regression, the impact of environmental variables on single- and multi-trait functional indices was examined. Prior to analysis, all environmental variables were standardised to enable beta-coefficient comparison of selected environmental variables within models. Model selection was based the Akaike Information Criterion (AIC) – a measure of goodness of fit of the data to estimated statistical model- with the model having the lowest AIC selected.

4.3. Results

4.3.1. The Impact of Environmental and Management Factors on the Functional Structure and Composition of Temperate Grasslands

The results of the series of one-way ANOVAs (Table-4.3.1.) demonstrate that there were significant differences in grazing statuses, turf heights, applications of farmyard manure and improvement statues in temperate grasslands of the National Vegetation Classification communities and sub-communities. Differences in grazing statuses were demonstrated in functional divergence at the multi- (MNND) and single-trait (plant height) level, as well as in the evenness of leaf thickness and the community-weighted means of plant height, specific leaf area, seed mass, leaf nitrogen content and leaf C:N. These effects are analysed further in 4.3.1a.

The application of farmyard manure was reported to influence functional divergence at the multi- and single-trait level – leaf dry matter content, specific leaf area, leaf thickness and leaf nitrogen content. These are analysed further in 4.3.1b. Improvement activities showed significant differences in functional richness, functional divergence, the multi-trait functional divergence as well as plant height, leaf dry matter content, specific leaf area and leaf nitrogen content – analysis in 4.3.1c.

Index	**Grazing Status**	**Farmyard Manure**	**Improvement Status**
	$F_{[df]}$	$F_{[df]}$	$F_{[df]}$
FRic	NS	NS	$14.77_{[2,24]}$***
FEve	NS	NS	$3.78_{[2,24]}$*
FDiv	NS	NS	NS
MNND	$3.57_{[2,71]}$*	$6.81_{[1,25]}$*	$6.43_{[2,24]}$*
CMW_{PH}	$11.83_{[2,71]}$***	$6.52_{[1,25]}$*	$7.83_{[2,24]}$*
CWM_{LDMC}	NS	NS	NS

CWM_{SLA}	$6.19_{[2,71]}$*	NS	NS
CWM_{Seed}	$15.01_{[2,71]}$***	NS	NS
CWM_{Thick}	NS	NS	NS
CWM_{LNC}	$10.57_{[2,71]}$***	$8.86_{[1,25]}$*	$15.46_{[2,24]}$***
$CWM_{C:N}$	$4.23_{[2,71]}$*	NS	$13.33_{[2,24]}$***
FRO_{SLA}	NS	NS	NS
FRO_{Seed}	NS	NS	NS
FRO_{Thick}	$5.14_{[2,71]}$*	$10.37_{[1,25]}$*	NS
FRO_{LNC}	NS	NS	NS
$Range_{PH}$	$5.81_{[2,71]}$*	NS	$5.06_{[2,24]}$*
$Range_{LDMC}$	NS	$15.27_{[1,25]}$***	$17.63_{[2,24]}$***
$Range_{SLA}$	NS	NS	$5.98_{[2,24]}$*
$Range_{Thick}$	NS	NS	NS
$Range_{LNC}$	NS	NS	NS
$Range_{C:N}$	NS	NS	NS
$MNND_{LDMC}$	NS	NS	NS
$MNND_{SLA}$	NS	$4.97_{[1,25]}$*	NS
$MNND_{Seed}$	NS	NS	NS
$MNND_{Thick}$	NS	$6.60_{[1,25]}$*	NS
$MNND_{LNC}$	NS	$11.91_{[1,25]}$*	$10.99_{[2,24]}$***
$MNND_{C:N}$	NS	NS	NS

*Table-4.3.1. F-values and significance levels (0.000= ***, 0.001 = **, 0.05 = *, NS = not significant) for the effect of grazing status (low intensity, aftermath grazing, grazed, application of farmyard manure (yes, no), and improvement status (improved, semi-improved and unimproved) of mesotrophic grasslands on single- and multi-trait functional diversity indices. Abbreviations: FRic, Functional Richness;*

FEve, Functional Evenness; FDiv, Functional Divergence; MNND, Mean Nearest Neighbour Distance; CWM, Community-Weighted Mean; PH, Plant Height; LDMC, Leaf Dry Matter Content; SLA, Specific Leaf Area; Seed, Seed Mass; Thick, Leaf Thickness; LNC, Leaf Nitrogen Content; C:N, Leaf C:N; FRO, Functional Regularity.

4.3.1a. The Effect of Grazing Status

A series of one-way ANOVAs were conducted to compare the effect of grazing on functional diversity indices for low intensity, aftermath and grazed NVC communities and sub-communities. There were significant grazing effects on MNND, CWM_{PH}, CWM_{SLA}, CWM_{Seed}, CWM_{LNC}, $CWM_{C:N}$, FRO_{Thick} and $Range_{PH}$ (Table-4.3.1.). Post Tukey HSD tests indicated that the mean scores of CWM_{PH} and CWM_{Seed} for low intensity grazing were significantly different from the aftermath grazing and grazed groups. However, the aftermath grazing and grazed groups did not significantly differ. Further post hoc comparisons using the Tukey HSD test indicated that the mean scores of CWM_{SLA}, CWM_{LNC}, $CWM_{C:N}$ and $Range_{PH}$ for the grazed condition were significantly different than the low intensity grazing condition. However, aftermath grazing did not significantly differ from the low intensity and regularly grazed conditions. Moreover, functional divergence (MNND) demonstrated that increasing grazing pressure resulted in a greater breadth of the niche space. The distribution of leaf thicknesses in the niche space, however, peaked in evenness with aftermath grazing.

	Grazing Status		
Index	*Low Intensity*	*Aftermath*	*Grazed*
MNND	1.71±0.03 **A**	1.8±0.05 **B**	1.96±0.03 **C**
CWM_{PH} (m)	0.62±0.03 **A**	0.48±0.02 **B**	0.4±0.01 **B**
$logCMW_{SLA}$ (mm^2/mg)	1.41±0.01 **A**	1.39±0.01 **A**	1.36±0.00 **B**
CWM_{Seed} (mg)	2.15±0.05 **A**	1.35±0.1 **B**	1.06±0.05 **B**
CWM_{LNC} (mg/g)	26.88±0.56 **B**	24.43±0.57 **B**	22.84±0.25 **A**

$CWM_{C:N}$	18.14±0.4 **B**	21.58±1.66 **B**	22.09±0.28 **A**
FRO_{Thick}	0.47±0.02 **A**	0.53±0.02 **B**	0.41±0.02 **A**
$Range_{PH}$	2.77±0.55 **A**	1.78±0.32 **B**	1.31±0.11 **B**

Table-4.3.2. Grazing status level averages ± standard errors for single- and multi-trait functional diversity indices analysed using ANOVA. Bolded letters note the Tukey HSD groupings. Abbreviations: MNND, Mean Nearest Neighbour Distance; CWM, Community-Weighted Mean; PH, Plant Height; SLA, Specific Leaf Area; Seed, Seed Mass; Thick, Leaf Thickness; LNC, Leaf Nitrogen Content; C:N, Leaf C:N; FRO, Functional Regularity.

4.3.1b. The Effect of Applying Farmyard Manure on Mesotrophic Grasslands

The use of farmyard manure was found to have a significant effect on MNND, CWM_{PH}, CWM_{LNC}, FRO_{Thick}, $Range_{LDMC}$,$MNND_{SLA}$, $MNND_{Thick}$ and $MNND_{LNC}$ (Figure-4.3.1. and Table-4.3.3.). The addition of farmyard manure was found to cause convergence at the multi-trait level. Adding farmyard manure was found to decrease CWM_{PH} and cause convergence in leaf nitrogen content. Convergence was also detected in specific leaf area and leaf thickness; leaf thickness was found to be more even with farmyard manuring. On the other hand, divergence in leaf dry matter content was found.

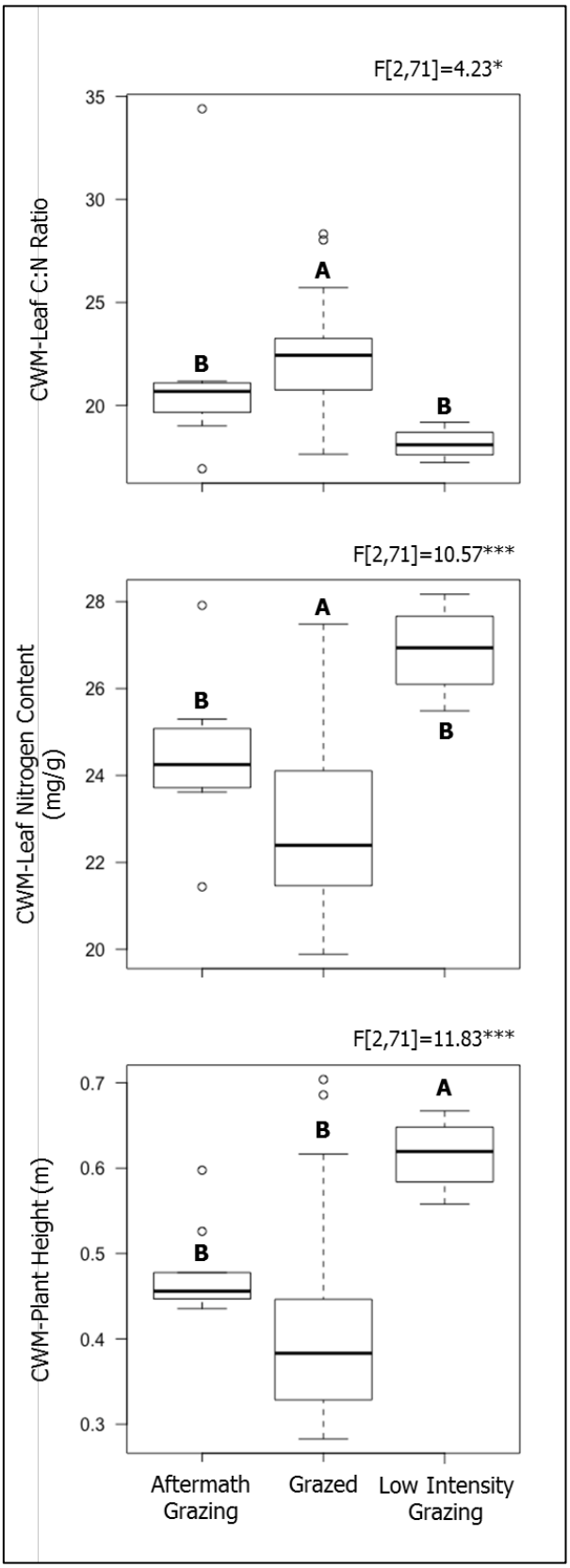

Figure-4.3.1: Boxplots comparing the effect of grazing status on community-weighted means of leaf C:N, leaf nitrogen content and plant height. Bolded letters note the Tukey HSD groupings. Abbreviation: CWM, Community-Weighted Mean.

	Farmyard Manure	
Index	*No*	*Yes*
logMNND	0.30±0.01	0.24±0.01
CWM_{PH} *(m)*	0.55±0.02	0.46±0.01
$logCWM_{LNC}$ *(mg/g)*	1.41±0.00	1.39±0.01
$logFRO_{Thick}$	-0.33±0.01	-0.26±0.01
$Range_{LDMC}$	0.25±0.01	0.29±0.01
$MNND_{SLA}$	0.57±0.05	0.33±0.08
$MNND_{Thick}$	0.01±0.00	0.00±0.00
$logMNND_{LNC}$	-2.50±0.03	-2.53±0.06

Table-4.3.3. the application of farmyard manure averages ± standard errors for single- and multi-trait functional diversity indices analysed using ANOVA. Abbreviations: MNND, Mean Nearest Neighbour Distance; CWM, Community-Weighted Mean; PH, Plant Height; LDMC, Leaf Dry Matter Content; SLA, Specific Leaf Area; Thick, Leaf Thickness; LNC, Leaf Nitrogen Content; FRO, Functional Regularity.

4.3.1c. The Effect of Improvement Status on Mesotrophic Grasslands

Improvement status (unimproved, semi-improved and improved) was found, from a series of one-way ANOVAs, to affect ten single and multi-trait functional diversity indices (Table-4.3.4.). Post hoc tests (Tukey HSD test) for the indices FRic, CWM_{LNC} revealed significant differences between each improvement group; as improvement progresses, niche space occupancy at the multi-trait level (FRic) was found to reduce and community-level leaf nitrogen content increase. Tukey's HSD test further elaborated that any improvement (semi-improved and improved) caused divergence at the multi-trait level (MNND) and for plant height ($Range_{PH}$), but leaf dry matter content ($Range_{LDMC}$) was found to converge. Temperate grasslands beyond semi-improvement were shown to have a reduced leaf C:N at the community-level ($CWM_{C:N}$), but divergence in leaf nitrogen content ($MNND_{LNC}$) was

apparent. Evenness at the multi-trait level (FEve) was found to dip with semi-improvement, whereas, community plant height (CWM_{PH}) and divergence of specific leaf area ($Range_{SLA}$) peaked.

	Improvement Status		
Indice	*Improved*	*Semi-Improved*	*Unimproved*
FRic	0.01±0.01 **A**	0.01±0.00 **A**	0.04±0.01 **B**
FEve	0.84±0.01 **A**	0.81±0.01 **B**	0.84±0.01 **A**
logMNND	0.32±0.01 **A**	0.29±0.01 **B**	0.24±0.01 **C**
CWM_{PH} *(m)*	0.52±0.02 **A**	0.58±0.02 **B**	0.46±0.01 **A**
$logCWM_{LNC}$ (mm^2/mg)	1.43±0.00 **A**	1.41±0.01 **B**	1.39±0.00 **C**
$logCWM_{C:N}$	1.25±0.00 **A**	1.29±0.01 **B**	1.31±0.02 **C**
$Range_{PH}$	2.99±0.36 **A**	2.47±0.3 **B**	1.44±0.26 **A**
$Range_{LDMC}$	0.24±0.00 **A**	0.24±0.01 **A**	0.29±0.01 **B**
$Range_{SLA}$	27.84±0.58 **A**	40.21±2.74 **B**	29.15±3.44 **A**
$logMNND_{LNC}$	-0.27±0.01 **A**	-0.31±0.02 **A**	-0.30±0.01 **B**

Table-4.3.4. improvement status level averages ± standard errors for single- and multi-trait functional diversity indices analysed using ANOVA. Bolded letters note the Tukey HSD groupings. Abbreviations: FRic, Functional Richness; FEve, Functional Evenness; MNND, Mean Nearest Neighbour Distance; CWM, Community-Weighted Mean; PH, Plant Height; LDMC, Leaf Dry Matter Content; SLA, Specific Leaf Area; LNC, Leaf Nitrogen Content.

4.3.1d. Summary of the Effects of Environmental and Management Factors on the Functional Structure and Composition of Temperate Grasslands

4.3.1di. Grazing Status

The results 4.2.1a and Figure-4.3.1 demonstrate that the influences of grazing status on functional structure and composition are apparent at the community/sub-community level of temperate grasslands. In essence, low intensity grazing produced swards of taller stature and larger seeds. Additionally, continually-grazed grassland communities converged with regards to plant height, selecting for shorter plants with lower values of specific leaf area and reduced leaf nitrogen content, and consequently greater levels of leaf C:N. The trait profile and convergence-divergence patterns are typical for grazed temperate grasslands; however, the novelty resides in the finding that responses of grazing tolerant species were found at low intensity grazing, but intensively grazed communities displayed a grazing avoidance strategy; aftermath grazing showed size characteristics (plant height and seed mass) of grazing avoidant species but foliar traits characteristic of grazing tolerant species. Despite this, divergence was still detected at the multi-trait level for intensively grazed communities.

	Low Intensity	*Aftermathe*	*Grazed*
MNND	Convergence ←	→	Divergence
CWM_{PH}	Tall ←	→ Short	
CWM_{Seed}	Heavy Seeds ←	→ Light Seeds	
$Range_{PH}$		Divergence ←	→ Convergence
CWM_{SLA}		Fast RGR ←	→ Slow RGR
CWM_{LNC}		High ←	→ Low
$CWM_{C:N}$		Low ←	→ High
FRO_{Thick}	Random	Even	Random

Figure-4.3.2. Summary of the results of the effect of grazing status on the functional structure and composition of NVC communities and sub-communities. Abbreviations: MNND, Mean Nearest Neighbour Distance; CWM, Community-Weighted Mean; PH, Plant Height; Seed, Seed Mass; SLA, Specific Leaf Area; LNC,

Leaf Nitrogent Content; C:N, Leaf C:N; FRO, Functional Regularity; Thick, Leaf Thcikness; RGR, Relative Growth Rate.

4.3.1dii. Agricultural Improvement

Agricultural improvement utilising inorganic and organic sources was found to influence a variety of single- and multi-trait indices. The contrasting effects of organic and inorganic fertilisation are highlighted in Figure-4.3.3. for the community-weighted mean of leaf nitrogen content and functional divergence at the multi-and single-trait level (leaf dry matter content and leaf nitrogen content); the application of farmyard was comparable to the unimproved status.

The findings further postulate that improvement levels (semi or improved) can have differing effects on functional diversity, for example, a status of "improved" produces a plant community that is diverged in plant height, leaf nitrogen content and leaf dry matter content. The functional divergence of specific leaf area was found at semi-improvement status and convergence at the two extremities (improved and unimproved). Divergence at the multi-trait level and in plant height was also detected with any improvement (semi or improved) together with an increase in the community-weighted mean of leaf nitrogen content and the subsequent decrease in leaf C:N.

	Improvement Status			*Farmyard Manure*	
	Improved	*Semi-Improved*	*Unimproved*	*Yes*	*No*
FRic		High ↔ Low			
Range$_{LDMC}$		Convergence ↔ Divergence		Divergence ↔	Convergence
MNND$_{LNC}$		Divergence ↔ Convergence		Convergence ↔	Divergence
MNND	Divergence ↔		Convergence	Convergence ↔	Divergence
CWM$_{LNC}$	High ↔		Low	Low ↔	High
CWM$_{C:N}$	Low ↔		High		
Range$_{PH}$	Divergence ↔		Convergence		
FEve	Random	Even	Random		
CWM$_{PH}$	Short	Tall	Shorter	Short ↔	Tall
MNND/Range$_{SLA}$	Convergence	Divergence	Convergence	Convergence ↔	Divergence
MNND$_{Thick}$				Convergence ↔	Divergence
FRO$_{Thick}$				Even ↔	Random

Figure-4.3.3. Summary of the results of the effect of improvement status and application of farmyard manure on the functional structure and composition of NVC communities and sub-communities. Abbreviations: FRic, Functional Richness; FEve, Functional Evenness; MNND, Mean Nearest Neighbour Distance; CWM, Community-Weighted Mean; PH, Plant Height; LDMC, Leaf Dry Matter Content; SLA, Specific Leaf Area; Thick, Leaf Thickness; LNC, Leaf Nitrogen Content; C:N, Leaf C:N.

The application of farmyard manure converged at the multi-trait level and for specific leaf area, leaf thickness and the leaf nitrogen content of the communities toward reduced levels of leaf nitrogen content. Leaf dry matter content, however, was found to diverge. Furthermore, the distributions of plant species and leaf thickness trait values were more even.

Together these findings indicate that the use of inorganic fertilisers stimulates plant responses typical of an exploitative ecological strategy, whereas farmyard manuring stimulates a conservative response that is analogous to unimproved grasslands.

4.3.2. Examining the Relationship between Plant Traits and Environmental Variables in the PGE (1991-2000)

The combined RLQ and fourth-corner analysis revealed significant relationships between plant functional traits and environmental descriptors through their associations with RLQ axes. Leaf thickness and leaf nitrogen content, together with pH, grass diversity and grass coverage were the plant traits and environmental variables that impacted species' distribution, given their relationships with RLQ axis two (Figure-4.3.7.). The fourth-corner statistic, examining the global relationship between species traits and environmental variables was significant ($p = 0.048$), and therefore reject the null hypothesis of Model 6 that species are distributed irrespective of their traits and/or environmental conditions.

RLQ axis two was significantly negatively correlated with pH and positively with grass diversity and coverage. Associated traits are thicker leaves with greater nitrogen content for more alkaline sites, with decreased coverage and diversity of grass species. These results, however, should be interpreted tentatively due to the weak relationships that the traits and environmental variables have with RLQ axis two – highest R^2 recorded at -0.31.

a	*AxcR1*	*AxcR2*
Plant Height (m)	-0.30	-0.04
Leaf Dry Matter Content (g/g)	-0.18	0.19
Specific Leaf Area (mm^2/mg)	-0.05	0.18
Seed Mass (mg)	-0.17	-0.21
Leaf Thickness (mm)	0.20	**-0.28**
Leaf Nitrogen Content (mg/g)	-0.13	**-0.24**
Leaf C:N Ratio	0.11	0.18

b	*AxcQ1*	*AxcQ2*
Fertilisation Status	-0.13	0.02
Total Fertiliser Application Rate (kg/ha/yr)	-0.28	0.04
Total Nitrogen Fertiliser Application Rate (kg/ha/yr)	-0.19	0.19
Ammonium Sulphate Variety	-0.14	-0.09
Sodium Nitrate Variety	-0.11	0.23
Mineral Addition Status	**-0.23**	0.00
Total Mineral Fertiliser Application Rate (kg/ha/yr)	-0.28	0.00
Triple Superphosphate Application Rate (kg/ha/yr)	**-0.25**	-0.04
Potassium Sulphate Application Rate (kg/ha/yr)	-0.26	-0.07
Sodium Sulphate Application Rate (kg/ha/yr)	-0.20	-0.08
Magnesium Sulphate Application Rate (kg/ha/yr)	-0.23	-0.04
Water Soluble Sodium Silicate Application Rate (kg/ha/yr)	-0.09	0.05
Liming Status	0.09	0.22
pH	-0.06	**-0.31**
Grass Species Richness	0.03	-0.22
Grass Species Diversity (D)	0.02	**0.18**
Grass Species Evenness (E)	0.04	0.16
Grass Species Coverage (%)	-0.15	**0.31**

Figure-4.3.4. Fourth-corner and RLQ results for the PGE. (a) Fourth-corner tests between the first two RLQ axes for environmental gradients (AxR1, AxR2) and traits. (b) Fourth-corner tests between the first two RLQ axes for trait syndromes

(AxQ1, AxQ2) and environmental variables. Relationships with RLQ axes one and two are tabulated with significant correlations (R^2) bolded.

4.3.4. Effects of Environmental Variables on the Functional Diversity Indices of the PGE Plots

Fifteen environmental variables were found to feature in the best two single predictors of each of the single- and multi-trait indices. The model that explained the greatest amount of variability was found to be for FRic, whereas the poorest was FRO_{Seed}; evenness measures (FEve and FRO) were the poorest models, as shown by their R^2 values. Triple super phosphate application and grass coverage only positively influenced community-weighted means and the evenness of traits. Contrastingly, liming status, grass species' evenness, and total nitrogen fertiliser application rate had negative impacts on multi-trait indices, and the community-weighted means, evenness and the functional divergence of single traits. Grass species' richness and diversity, fertilisation and mineral addition status, magnesium sulphate, potassium sulphate and sodium sulphate application rate, total fertiliser application rate, mineral addition status, pH, and ammonium sulphate variety were found to have both mixed influences on the functional structure and composition of the PGE.

4.3.4a. The Impact at the Multi-Trait Level

The total variation explained by the multi-trait functional diversity indices ranged from 22% to 83%. Functional evenness was the most poorly explained index followed by FDiv. In essence, multi-trait functional diversity indices were ranked as functional richness>between-site functional divergence>within-site functional divergence>functional evenness.

The results from the multi-trait indices demonstrate that overall fertilisation (fertilisation status and total fertiliser application rate) has a negative impact on the occupancy (FRic) and breadth (FDiv and MNND) of niche spaces. However, the chemical composition of the fertiliser dictates the nature of the relationship; nitrogen-based fertilisers (total nitrogen fertiliser application rate and ammonium

sulphate variety) reduced the evenness (FEve) and breadth of the niche space (FDiv), whereas mineral based fertilisers (magnesium sulphate, sodium sulphate, and potassium sulphate) had positive effects on FEve, FDiv, and MNND. Taxonomic measures of grass species were found to be the strongest influences on the occupancy, evenness, and breadth of the niche space. Grass species' richness and diversity positively affected FRic, FEve, and MNND, whereas grass species' evenness showed the opposite effect on FEve and MNND. Increasing alkalinity of the PGE was found to have a negative effect on the occupancy of niche spaces (FRic) and the between-site functional divergence (βRao's Q).

Indice	**$F_{[df]}$**	**R^2**	**Positive Influences**	**Negative Influences**
FRic	$38.08_{[86,574]}$***	0.83	Grass Richness: 0.09±0.02	Fertilised: -0.06±0.02 Lime Addition: -0.02±0.00
FEve	$17.90_{[12,648]}$***	0.22	Grass Diversity: 0.29±0.04 Mg Addition: 0.04±0.01	Grass Evenness: -0.30±0.04 Nitro Fert App Rate: -0.20±0.09
FDiv	$17.40_{[60,600]}$***	0.59	K Addition: 5.77±2.90 Na Addition: 5.17±2.60	Tot Fert App Rate: -10.01±5.05 Ammonium: -4.35±2.21
MNND	$29.36_{[67,593]}$***	0.74	Grass Diversity: 2.30±0.31 K Addition:	Tot Fert App Rate: -1.79±0.62 Grass Evenness:

			0.93±0.35	-1.58±0.29
βRao's Q	$29.56_{[86,574]}$***	0.79	Minerals: 0.03±0.01	pH: -0.05±0.02 Grass Richness: -0.05±0.02

Table-4.3.5. Models as found by the stepwise linear regressions summarising the F-value [degrees of freedom], R^2, and the top two (where applicable) single positive and negative influences on multi-trait indices. Abbreviations: FRic, Functional Richness; FEve, Functional Evenness; FDiv, Functional Divergence; MNND, Mean Nearest Neighbour Distance; Mg Addition, Magnesium Sulphate; Nitro Fert App Rate, Total Nitrogen Fertiliser Application Rate; K Addition, Potassium Sulphate; Na Addition, Sodium Sulphate; Tot Fert App Rate, Total Fertiliser Application Rate; Ammonium, Ammonium Sulphate.

4.3.4b. The Impact on Community-Weighted Means

Indice	**$F_{[df]}$**	**R^2**	**Positive Influences**	**Negative Influences**
CWM_{PH}	$34.91_{[69,591]}$***	0.78	K Addition: 11.28±1.78 Na Addition: 10.08±1.59	Tot Fert App Rate: -19.48±3.09 Ammonium: -8.51±1.35
CWM_{LDMC}	$14.05_{[77,583]}$***	0.60	P Addition: 0.08±0.04 Grass Diversity: 0.06±0.01	Grass Evenness: -0.05±0.01 Minerals: -0.01±0.00
CWM_{SLA}	$18.12_{[69,591]}$***	0.64	K Addition: 104.70±29.61	Tot Fert App Rate: -180.53±51.56

			Na Addition: 93.35±26.54	Ammonium: -78.87±22.51
CWM$_{Seed}$	$15.82_{[77,583]}$***	0.63	Tot Fert App Rate: 6.63±0.81 Ammonium: 3.14±0.43	Na Addition: -3.54±0.51 K Addition: -2.96±0.45
CWM$_{Thick}$	$19.46_{[88,572]}$***	0.71	Tot Fert App Rate: 2.32±0.79 Ammonium: 1.01±0.35	K Addition: -1.35±0.46 Na Addition: -1.20±0.41
CWM$_{LNC}$	$23.48_{[76,572]}$***	0.72	Tot Fert App Rate: 3.96±0.41 Ammonium: 1.94±0.68	Nitro Fert App Rate: -1.89±0.43 Na Addition: -1.87±0.77
CWM$_{C:N}$	$21.87_{[74,584]}$***	0.70	Grass Coverage: 0.84±0.24 Minerals: 0.29±0.24	K Addition: -1.49±0.17 pH: -0.51±0.07

Table-4.3.6. Models as found by the stepwise linear regressions summarising the F-value [degrees of freedom], R^2, and the top two (where applicable) single positive and negative influences on community-weighted mean. Abbreviations: CWM, Community-Weighted Mean; PH, Plant Height; LDMC, Leaf Dry Matter Content; SLA, Specific Leaf Area; Seed, Seed Mass; Thick, Leaf Thickness; LNC, Leaf Nitrogen Content; C:N, Leaf C:N; K Addition, Potassium Sulphate; Na Addition; Sodium Sulphate; Tot Fert App Rate, Total Fertiliser Application Rate; Ammonium, Ammonium Sulphate; P Addition, Triple Superphosphate; Nitro Fert App Rate, Nitrogen Fertiliser Application Rate.

The findings in Table-4.3.6. highlight that climate and environmental variables were able to explain between 60% and 78% of the variation in community-weighted means of seven traits. Plant height recorded the highest R^2 (0.78) and leaf dry matter the lowest. The ranking of each trait is plant height, leaf nitrogen content, leaf thickness, leaf C:N, specific leaf area, seed mass and leaf dry matter content.

The community-weighted means of the seven traits demonstrate that fertilisation, particularly with ammonium sulphate, produces swards that are shorter with larger seeds, and thicker leaves with reduced specific leaf area. Mineral fertilisers, such as potassium sulphate and sodium sulphate, showed contrasting relationships, resulting in taller communities with smaller seeds, and leaves that were thinner and had larger specific leaf area. Sodium sulphate addition was also found to negatively impact leaf nitrogen content; potassium sulphate addition reduced leaf C:N ratio at the community level, whereas, triple superphosphate application increased leaf dry matter content. Biotic factors, pertaining to grass species' taxonomic measures, were less of an influence than inorganic fertilisers. Grass species' diversity positively affected leaf dry matter content at the community level but grass species' evenness had a lesser but negative impact. Grass species' coverage was also found to increase community-weighted leaf C:N. Finally increasing the alkalinity of soils was found to be negatively associated with community-level leaf C:N.

4.3.4c. The Impact on the Evenness of Single Traits

The R^2 values in Table-4.3.7. highlight that functional regularity/evenness of single traits was poorly explained; 21-41% of total variation was explained by environmental factors. A general ranking showed leaf thickness>specific leaf area>leaf nitrogen content>leaf C:N>seed mass.

Indice	**$F_{[df]}$**	**R^2**	**Positive Influences**	**Negative Influences**
FRO_{SLA}	$9.61_{[38,622]}$***	0.33	Tot Fert App Rate: 0.28±0.12 Grass Diversity: 0.26±0.03	Grass Evenness: -0.29±0.04 K Addition: -0.17±0.07
FRO_{Seed}	$3.17_{[84,576]}$***	0.21	Grass Diversity: 0.28±0.10 pH: 0.03±0.01	Grass Evenness: -0.32±0.10
FRO_{Thick}	$6.44_{[84,576]}$***	0.41	Tot Fert App Rate: 0.60±0.17 Ammonium: 0.22±0.09	K Addition: -0.32±0.09 Nitro Fert App Rate: -0.28±0.11
FRO_{LNC}	$6.98_{[40,620]}$***	0.26	Grass Diversity: 0.42±0.08 K Addition: 0.07±0.01	Grass Evenness: -0.42±0.08 Tot Fert App Rate: -0.09±0.01
$FRO_{C:N}$	$19.85_{[11,649]}$***	0.22	Grass Diversity: 0.29±0.03 P Addition: 0.03±0.01	Grass Evenness: -0.29±0.03 Minerals: -0.05±0.01

Table-4.3.7. Models as found by the stepwise linear regressions summarising the F-value [degrees of freedom], R^2, and the top two (where applicable) single positive and negative influences on evenness of single traits. Abbreviations: FRO, Functional Regularity; SLA, Specific Leaf Area; Seed, Seed Mass; Thick, Leaf Thickness; LNC,

Leaf Nitrogen Content; C:N, Leaf C:N; Tot Fert App Rate, Total Fertiliser Application Rate; K Addition, Potassium Sulphate; Ammonium, Ammonium Sulphate; Nitro Fert App Rate, Nitrogen Fertiliser Application Rate; P Addition, Triple Superphosphate.

Biotic factors, particularly grass species' diversity, and evenness, were found to have the strongest impacts on the evenness of specific leaf area, seed mass, leaf nitrogen content and leaf C:N; grass species' diversity having a positive effect and evenness a negative one. Communities with a more even distribution of grass species were found to reduce the evenness of the communities in terms of seed mass, specific leaf area, leaf nitrogen content and leaf C:N; grass species' diversity showed complete contrasting relationships. The evenness of leaf thickness, however, was more influenced by total fertiliser application rate, nitrogen fertiliser application rate, and ammonium sulphate variety. Ammonium sulphate variety was the strongest positive single factor influencing the evenness of leaf thickness in the swards. Finally, increasing alkalinity was found to have a positive impact the functional regularity of seed mass.

4.3.4d. The Impact on the Convergence/Divergence of Single Traits

Indice	**$F_{[df]}$**	**R^2**	**Positive Influences**	**Negative Influences**
$Range_{PH}$	$24.06_{[87,573]}$***	0.75	Grass Richness: 0.18±0.04 pH: 0.05±0.03	Lime Addition: -0.04±0.01
$Range_{LDMC}$	$23.73_{[74,586]}$***	0.72	Grass Diversity: 0.07±0.02 Grass Richness: 0.03±0.01	Grass Evenness: -0.07±0.02 Lime Addition: -0.01±0.00

$Range_{SLA}$	$49.61_{[40,620]}$***	0.74	Tot Fert App Rate: 33.33±4.35 Ammonium: 15.79±1.98	Na Addition: -17.37±2.37 K Addition: -16.86±2.45
$Range_{Thick}$	$20.91_{[76,584]}$***	0.69	Grass Richness: 0.04±0.01 K Addition: 0.02±0.01	Tot Fert App Rate: -0.03±0.01 Fertilised: -0.01±0.00
$Range_{LNC}$	$23.20_{[114,546]}$***	0.79	pH: 1.70±0.24	Minerals: -1.37±0.44 Fertilised: -1.11±0.23
$Range_{C:N}$	$19.67_{[123,537]}$***	0.78	Grass Richness: 2.10±0.69	Fertilised: -1.55±0.14 Mg Addition: -0.50±0.22
$MNND_{LDMC}$	$41.43_{[53,607]}$***	0.76	Grass Diversity: 0.02±0.01	
$MNND_{SLA}$	$14.35_{[59,601]}$***	0.54	K Addition: 37.08±12.94 Na Addition: 33.38±11.61	Tot Fert App Rate: -64.28±22.53 Ammonium: -28.29±9.84
$MNND_{Seed}$	$21.82_{[22,638]}$***	0.40	K Addition: 10.75±3.05	Tot Fert App Rate: -18.48±5.30

			Na Addition: 9.56±2.73	Ammonium: -8.12±2.32
$MNND_{Thick}$	$22.54_{[57,603]}$***	0.65	Grass Diversity: 0.01±0.00	Grass Evenness: -0.01±0.00
$MNND_{LNC}$	$9.54_{[75,585]}$***	0.49	Grass Diversity: 1.11±0.35 Fertilised: 0.11±0.02	Mg Addition: -0.11±0.03
$MNND_{C:N}$	$16.87_{[67,593]}$***	0.61	Na Addition: 1.14±0.35 K Addition: 1.03±0.33	Tot Fert App Rate: -1.85±0.59 Ammonium: -0.97±0.30

Table-4.3.8. Table-4.3.6. Models as found by the stepwise linear regressions summarising the F-value [degrees of freedom], R^2, and the top two (where applicable) single positive and negative influences on the functional divergence of single traits. Abbreviations: MNND, Mean Nearest Neighbour Distance; ; PH, Plant Height; LDMC, Leaf Dry Matter Content; SLA, Specific Leaf Area; Seed, Seed Mass; Thick, Leaf Thickness; LNC, Leaf Nitrogen Content; C:N, Leaf C:N; Tot Fert App Rate, Total Fertiliser Application Rate; Ammonium, Ammonium Sulphate; Na Addition, Sodium Sulphate; K Addition, Potassium Sulphate.

The total variation explained in functional divergence at the single-trait level ranged from 40% to 78%. Generally, the range was better explained by abiotic and biotic factors than the mean nearest neighbour distance measure of functional divergence. A ranking of the R^2 values was found to be leaf thickness>leaf dry matter content>specific leaf area>plant height>leaf C:N>leaf nitrogen content. The functional divergence of seed mass was the poorest explained plant trait.

Grass species' diversity and evenness were found to have the strongest impacts on the divergence of plant height, leaf dry matter content, leaf thickness and leaf C:N; grass species' richness and diversity having positive effects on the divergence of these traits and grass species' evenness having strong negative impacts on leaf dry matter content and leaf thickness. Fertilisation, particularly with ammonium sulphate, was shown to cause convergence in seed mass and leaf C:N, however, the application of mineral fertilisers (potassium sulphate and sodium sulphate) are shown to cause divergence in leaf thickness, seed mass, and leaf C:N.

4.3.5. Key Results from the Analyses of the Park Grass Experiment

The results of the combined RLQ and fourth-corner analysis and the stepwise linear regressions shed light on the impacts of abiotic and biotic factors on temperate grasslands. Particular insights were revealed about the influences of different fertiliser varieties and the coverage and taxonomic features of the standing grass species on the functional structure and composition of the Park Grass Experiment. These relationships are summarised in Figure-4.3.8. and analysed further in 4.3.5a. and 4.3.5b.

4.3.5a. Abiotic Factors: Fertiliser Variety and Soil pH

Fertilisation of the Park Grass Experiment affected the functional structure and composition at the multi- and single-trait level (specific leaf area, seed mass, leaf thickness and leaf nitrogen content). The patterning of functional structure and composition closely aligned with the application of ammonium sulphate. Niche occupancy and breadth decreased production of swards of short stature and heavier seeds that grow slowly and produce thin leaves rich in leaf nitrogen content. Despite the detection of convergence at the multi-trait level, ammonium sulphate fertilisation caused divergence in specific leaf area. Altogether, these findings suggest that long-term application of ammonium sulphate generates swards that adhere to the conservative ecological strategy.

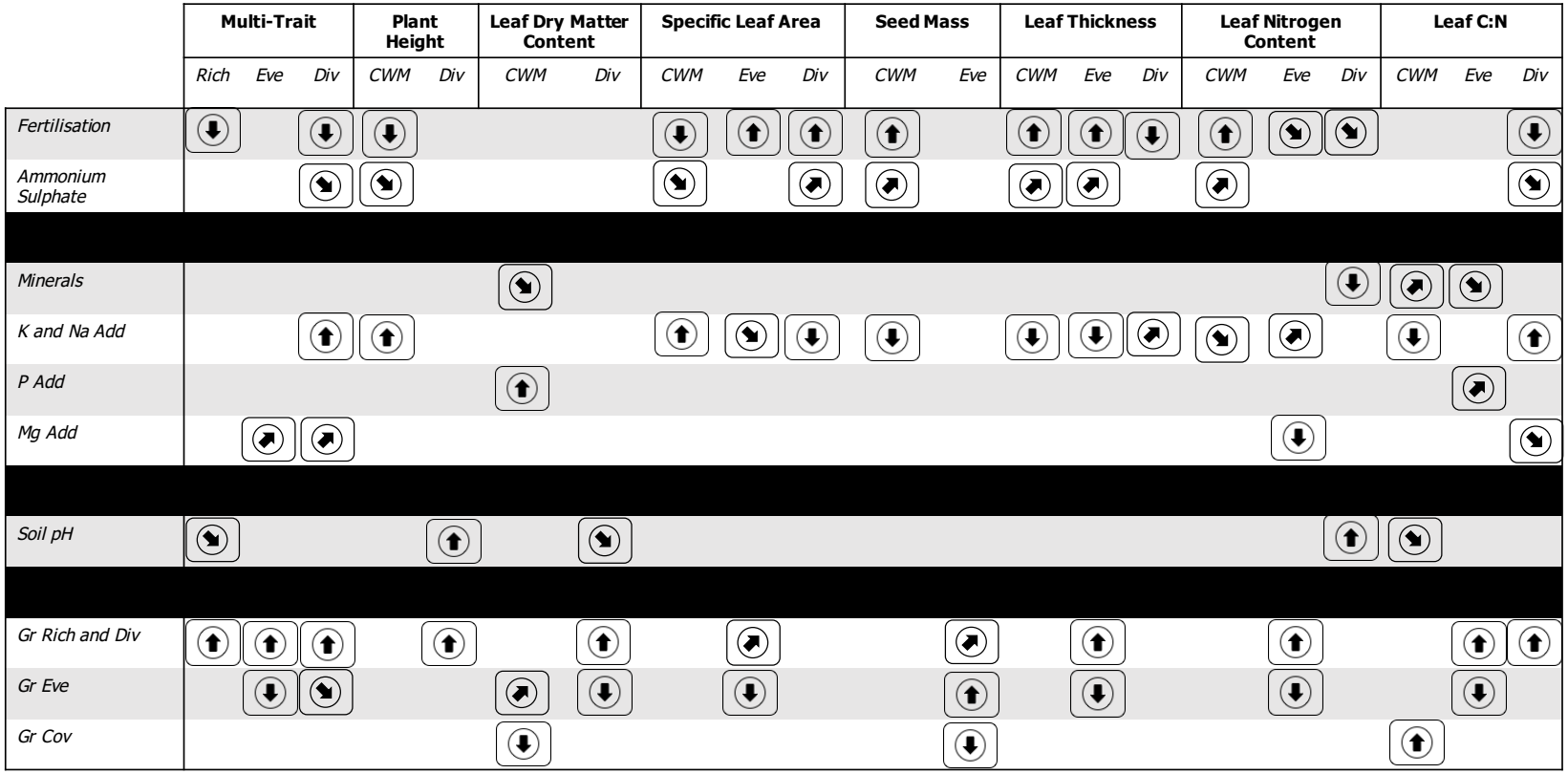

Figure-4.3.5. Environmental variables (abiotic and biotic) and their impacts on single- and multi-trait indices based on the analysis of the Park Grass Experiment reported in the main text. Upward pointing arrows indicate a positive effect, downward pointing arrows designate a negative effect. Abbreviations: Rich, Richness; Eve, Evenness: Div, Divergence; CWM, Community-Weighted Mean; K add, Potassium Sulphate; Na add, Sodium Sulphate; P add, Triple Superphosphate; Mg add, Magnesium Sulphate; Gr Rich and Div, Grass Species' Richness and Diversity; Gr Eve, Grass Species' Evenness.; Gr Cov, Grass Species' Coverage.

The application of ammonium sulphate has been associated with increasing acidity of the edaphic environment. Figure-4.3.8. provides evidence for this and suggests liming can increase the occupancy of the niche space at the multi-trait level, whilst causing convergence in plant height and leaf nitrogen content and increases in leaf C:N. It, therefore, could be deduced that fertiliser variety and soil acidity complement each other with regards to selecting the optimal ecological strategy – conservatism. However, the results of the RLQ-fourth corner analysis also postulate increasing soil alkalinity favouring conservative plant species. It is likely the extremities of soil pH mandate the adoption of the conservative strategy, whereas neutrality aligns with the exploitative strategy.

On the other hand, the application of mineral fertilisers, predominantly potassium sulphate and sodium sulphate, stimulates a plant species' response comparable to the exploitative ecological strategy. Niche breadth increased producing taller communities with light seeds that germinate and grow quickly developing thin leaves with reduced leaf C:N.

4.3.5b. Biotic Factors: Coverage and Taxonomy of Grass Species

Grass species predominantly affected the evenness of divergence of single-traits. Grass species' richness and diversity were found to directly contrast to grass species' evenness across a number of single-traits except for seed mass. Increasing richness and diversity induced divergence in plant height, leaf dry matter content, seed mass and leaf C:N suggesting grass species are a major component of the biotic filter in temperate grasslands. Grass species promote the functional divergence in traits due to competition – 'limiting similarity' hypothesis. Functional richness was congruent with grass species' richness and diversity. Evenness was found to increase with grass species richness and diversity, postulating that there is evidence of balanced coexistence and stability of trait values in the system through niche differentiation underpinned by foliar traits. However, there is evidence that grass species can act as a convergent force, especially when the distribution is even, such as in a monopolised community. Grass species' evenness induced convergence at the multi-trait level and for leaf dry matter content, whilst reducing

evenness at the multi-trait and single-trait level (specific leaf area, leaf thickness, leaf nitrogen content and leaf C:N).

Together, these results demonstrate the duality of the biotic filter that is dictated by the competition dynamics underpinned by complexity and distribution of grass species in a temperate grassland.

4.4. Discussion

The functional structure and composition of temperate grasslands are thought to be governed by abiotic and biotic filters underpinned by plant response traits (Lavorel and Garnier, 2002). In essence, the abiotic filter converges plant traits towards fitness optima that define a species' ability to grow, survive and reproduce, and thus represent the species' unique fundamental niche (Violle and Jiang, 2009). The biotic filter stimulates response trait divergence through the 'limiting similarity' hypothesis, which postulates that less effective competitors differentiate in their response traits/fundamental niche to co-exist (Funk *et al.*, 2008). Ecological response strategies have been widely distinguished at macro-scales and for a limited number of abiotic factors, such as soil nitrogen. However, criticisms lie in the applications of these strategies to different abiotic factors and at intermediate spatial scales. This chapter aimed to clarify the convergence-divergence paradox integral to the Response-Effect Framework whilst investigating previously under-studied abiotic factors (mineral fertilisers and intensity of grazing). Three hypotheses were proposed and each of these is discussed with reference to the key results (outlined in 4.3.1f. and 4.3.5) in the coming sections.

4.4.1. Grazing Regimes of Temperate Grasslands

The grazing tolerance and avoidance concepts have been confirmed at global and fine spatial scales, and a core list in relation to size and leaf physiology and chemistry has evolved (Díaz, Noy-Meir and Cabido, 2001; Kahmen and Poschlod, 2004; Díaz, Sandra Lavorel, *et al.*, 2007; Lloyd *et al.*, 2010). The generalisability of grazing ecological strategies was questioned with regards to differential grazing intensities and spatial scales. This chapter demonstrated that the ecological strategies suggested for plant trait responses to grazing hold at the regional and national level using the National Vegetation Classification data. This chapter hypothesised that increasing grazing intensity will cause convergence in plant traits typical of the grazing tolerance strategy; the findings satisfied this hypothesis and provided further elaboration significant to the management and productivity of temperate grasslands.

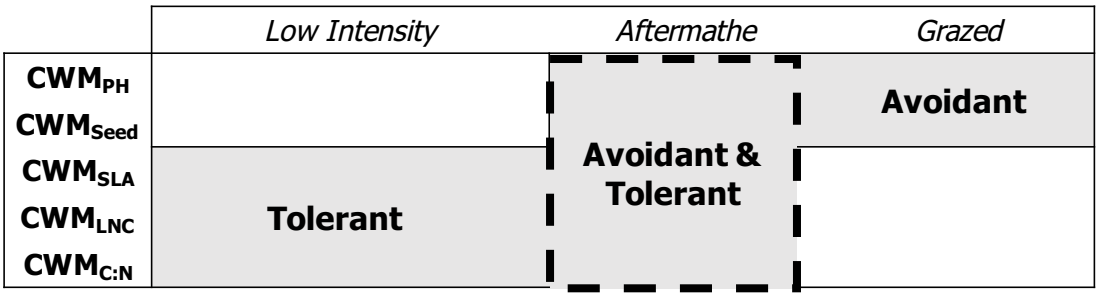

Figure-4.4.1. Summary of the patterning and overlap between avoidant and tolerant strategies to differing grazing intensities using community-weighted means. Abbreviations: CWM, Community-Weighted Mean; PH, Plant Height; Seed, Seed Mass; SLA, Specific Leaf Area; LNC, Leaf Nitrogen Content; C:N, Leaf C:N.

The segregation of three grazing intensities (low intensity, aftermath, and intensive grazing) and analysis of community-weighted means revealed that intensively grazed swards displayed grazing avoidance traits, and low intensity grazed swards tolerant traits. However, aftermath grazing supported a mosaic of traits that resemble both tolerance in leaf traits and avoidance in size traits (Figure-4.4.1.). This supports the findings of da Pontes *et al* (2010) for tolerant and avoidance responses of pasture grasses to cutting frequency – the reduction in a number of leaves and plant height. Grazing strategies, therefore, are not circumscribed to these binary responses (tolerant or avoidant) and a grazing response continuum is likely. The tolerance and avoidance strategies are suggested and illustrated to have fitness optima and tipping points in Figure-4.4.2.

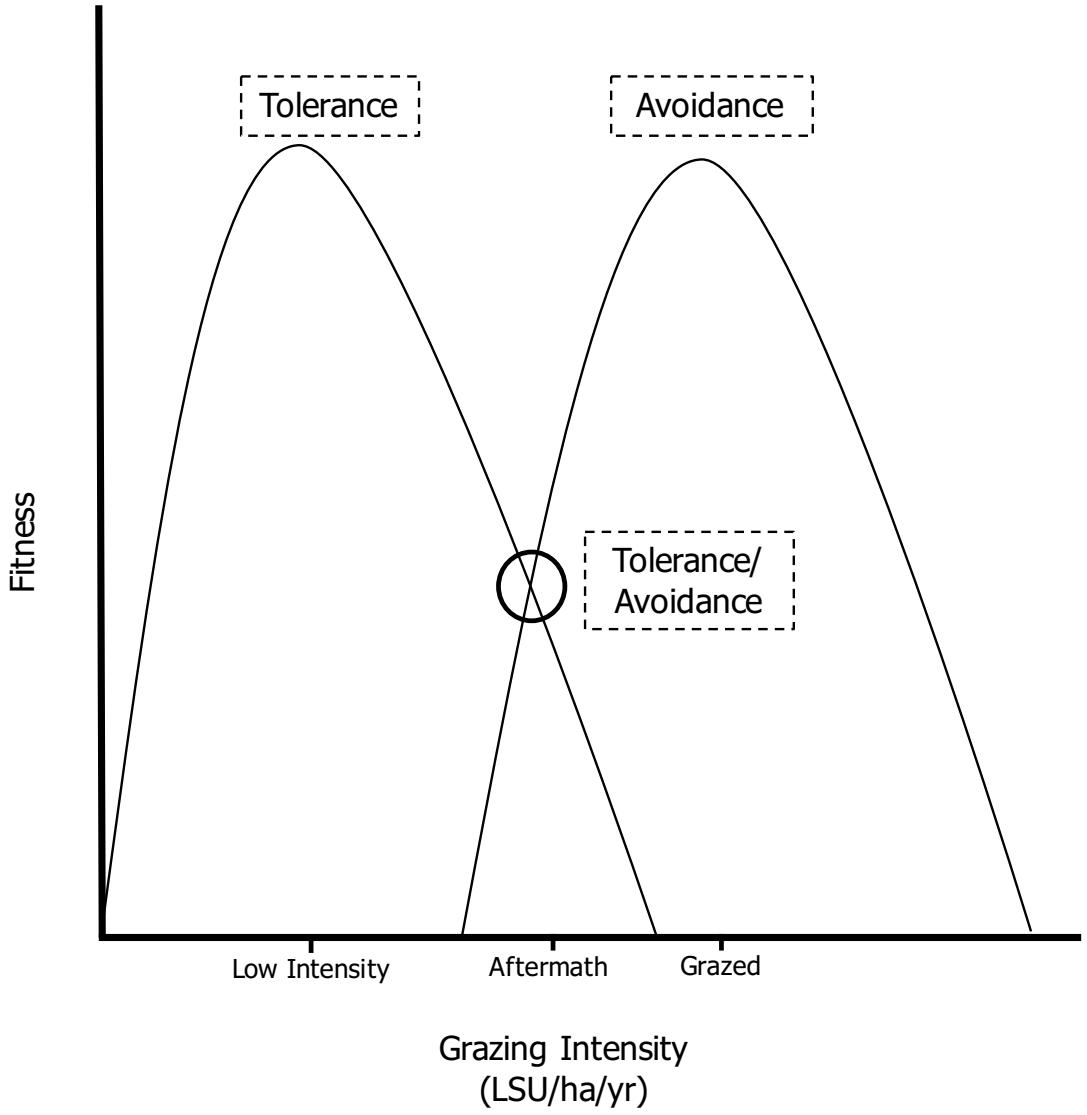

Figure-4.4.2. Graphical representation of the fitness optima for tolerant and avoidance strategies in relation to grazing intensity. Regions are indicated the highlight fitness optima for each strategy and the overlap of tolerance/avoidance with aftermath grazing activities.

The trait profiles of the grazing tolerant and avoidance strategies found in this chapter are congruent with the previous literature. The finding of increased specific leaf area and leaf nitrogen content with grazing tolerance echoes the research of Kahmen and Poschlod (2004), which associated grazing tolerance positively with leaf relative growth rates and leaf palatability. Leaf palatability and size traits was also found to be associated with grazing avoidance and reiterates Lloyd *et al* (2010) who suggested that avoidance is typified by evasion of defoliation through smaller plant stature and dormancy of seeds, together with slow-growing plant organs of

low palatability. This provides evidence for the validity of general trait-based ecological strategies and restates Díaz's emphasis on understanding communities' responses to grazing intensity and frequency. Future research should focus on defining a spectrum of plant trait responses with a greater degree of complexity, accounting for grazing frequency and intensity together with grazer species. The existence and study of grazer-exclusive plant strategies is rudimentary but definition of these could have important implications for the husbandry of livestock and maintenance or restoration of biodiversity.

This chapter inferred the conservation of biodiversity through the three components of functional diversity; richness, evenness, and divergence (Villéger, Mason and Mouillot, 2008). In essence, these elements classify the occupancy, distribution, and breadth of the niche space, which encapsulate the intrinsic resilience and stability of the community through functional redundancy. A larger and diverse niche space with an even distribution is assumed to guarantee longevity and maximise resource utilisation. Traditionally, grazing was considered a convergent force, which narrows plant responses in temperate grasslands towards tolerance (Díaz, Sandra Lavorel, *et al.*, 2007). This chapter identified support for this claim at the single trait level whereby plant height was found to converge towards a smaller stature at the highest grazing intensity; however, at the multi-trait level, divergence was reported. This posits the presence of a divergence pressure succeeding the convergent filtering of plant height, which Lavorel and Garnier (2002) speculated was the product of the biotic filter derived from interspecific competition dynamics.

Divergence is underpinned by the 'limiting similarity' hypothesis. In a finite niche space, the best competitors occupy the majority of the niche space driving subordinates to differentiate to co-exist (Funk *et al.*, 2008). Divergence in plant height is at the forefront of trait-based competition dynamics with reference to solar radiation capture and use, and is often integrated into defining competitive hierarchies and associations with species' competition coefficients (Park, Benjamin and Watkinson, 2010; Shipley *et al.*, 2011). The results of grazing responses contradict the systematic use of plant height in the literature, as in intensively grazed grasslands divergence in plant height was not detected in this chapter.

Logically, light resources in continually grazed grasslands are abundant, given the lack of canopy and rate of defoliation, therefore in these systems, divergence is speculated to be determined by traits associated with other limiting factors, such as nutrient or water resources. Interspecific competition for these resources has been established in leaf and root traits; the latter was omitted in the analyses of this chapter thus pointing to the significance of leaf traits despite the failure to identify functional divergence in them.

An alternative explanation proposes herbivory as a divergent force at the multi-trait level. Herbivory can induce spatial heterogeneity through trampling and deposition of urine and faeces. This diversifies the range of niche spaces through offering microsites that differ in soil moisture status and nutrient pools. This creates new germination and establishment niches that drive divergence in plant traits to capture and use the influx of resources (Doll *et al.*, 2011; De Bello *et al.*, 2013). Under the assumption of functional diversity, system resiliency and productivity are speculated to be high, yet upon coupling this with the results of the community-weighted means one could postulate that intensive grazing has created a rank grassland. This is evident in the community-weighted means of low specific leaf area, leaf nitrogen content and high leaf C:N and the uneven distribution of leaf thickness in the niche space. This postulation emphasises the importance of including single-trait functional diversity indices into response trait investigations and highlights the problem of abstraction and false conclusions drawn from multi-trait indices.

4.4.2. Improvement Status of Temperate Grasslands

Plant trait-based responses along nutrient gradients are thought to be phylogenetically conserved and has focussed on the leaf economics spectrum (Díaz *et al.*, 2004; Wright *et al.*, 2004). The trait profile of the leaf economics spectrum has been translated into separate ecological strategies defining plant species as exploitative or conservative. Research has defined the trait composition for N-exploitative and N-conservative plant types for low-mountain grasslands and a general consensus emerged that responses to other soil nutrients, such as phosphorus, co-vary with responses observed N-exploitative and conservative plant

types (Maire *et al.*, 2009). This chapter hypothesised that the traits and their convergence/divergence patterns of improved temperate grassland would resemble those defined by Maire *et al* (2009), irrespective of organic (farmyard manure) or inorganic (artificial fertilisers) origin. The results confirmed the adoption of the exploitative strategy in response to the general improvement of temperate grasslands, however, the application of farmyard manure stimulated plant trait responses typical of the conservative strategy.

The community-weighted mean of leaf nitrogen content was central to the finding of inorganic-exploitative and organic-conservative strategies; high and low leaf nitrogen content with improved grasslands and application of farmyard respectively. Schellberg and Pontes (2012) highlighted leaf nitrogen content as a core plant response trait to nitrogen supply and research has associated high leaf nitrogen content with high relative growth rates, high leaf photosynthetic rate, high root uptake capacity, reduced leaf dry matter content and low leaf N use efficiency (Tjoelker *et al.*, 2005). Conservative species coincide with the antithesis. This chapter uniquely postulates the differential effects of inorganic and organic fertilisation on exploitative vs conservative strategies but also provides insights into convergence/divergence patterns.

Under the assumption of abiotic filtering, increasing soil improvement status and application of farmyard manure are thought to stimulate convergence at the multi-trait level (Harpole and Tilman, 2007). However, the occupancy and breadth of temperate grassland niche spaces were found to increase with improvement status and converge with the farmyard manuring. The application of farmyard manure illustrates the expected convergence at the multi- and single-trait level with decreases in the ranges of specific leaf area, leaf nitrogen content and leaf thickness. Increased functional evenness and divergence, however, were demonstrated for leaf thickness and leaf dry matter content respectively. One could surmise that the application of farmyard manure triggers interspecific competition underpinned by a species' ability to survive; leaf dry matter content commonly aligns with leaf tissue density and leaf life span (Ryser and Lambers, 1995). The divergence of leaf dry matter content and evenness of leaf thickness signifies the

greater breadth and utilisation of temporal niches. Temporal niche differentiation enables species to access a shared resource for a limited period with reduced interspecific competition (Chesson, 2000). This guarantees the long-term sustainability of the community through continual niche occupation and habitation, thus maintaining ecosystem processes, functions, and services.

On the other hand, the improvement of temperate grasslands resulted in divergence in plant height and leaf nitrogen content. As with the grazing response, this divergence is speculated to be exemplary of the biotic filter and the 'limiting similarity' hypothesis and supports the assumed role of plant height in driving interspecific competition – especially in inorganic fertilised environments (Benjamin and Park, 2007). Much like, Section 4.4.1., the sole use of multi-trait indices should be limited, as nonsensical conclusions could be made; improvement is a weak abiotic filter that produces temperate grasslands that are resilient to climate and environmental variations due to the increases in functional richness and divergence. A history of intensive agricultural improvement has left 79% of temperate grasslands in the United Kingdom in an unfavourable condition and much of this has been attributed to the abuse of inorganic fertilisers in the late 1960's to early 1980's (Bullock *et al.*, 2011).

4.4.3. The Significance of Fertiliser Type

Research has heavily focussed on response traits along the soil nitrogen gradient under the assumption that soil nutrients co-vary along this gradient, hence the exploitative vs conservative strategy has become archetypal. Different types of nitrogen-based fertilisers (ammonium-based and nitrate-based) have been shown to induce niche differentiation in grass species, elucidated by changes in root uptake capacity, to promote co-existence. Dominant grass species favoured nitrate-derived nitrogen and subordinate species ammonium-derived nitrogen (da Silveira Pontes *et al.*, 2015). In contrast to da Silveira Pontes *et* al (2015), this chapter hypothesised that regardless of fertiliser type, plant trait responses will demonstrate a convergence towards a nitrogen-exploitative strategy. The findings elicited two plant trait responses that were distinct from what was originally hypothesised. General fertilisation and the application of ammonium sulphate converged response

traits towards a conservative strategy and interspecific competition drove plant species to inhabit different temporal niches. The application of mineral fertilisers (potassium sulphate and sodium sulphate) increased the breadth of response traits whilst selecting for exploitative species due to the alleviation of minerals as limiting resources. Consequently, the biotic filter generated divergence in plant traits responsible for the capture of light resources.

4.4.3a. Fertilisation and Ammonium Sulphate

The response traits of the dominant species and the convergence/divergence patterns were complementary for the findings on general fertilisation and the use of ammonium sulphate. The community-weighted means of specific leaf area and leaf thickness were found to decrease and increase respectively – characteristic of the conservative strategy. Short-term experiments have revealed the positive relationship between leaf nitrogen content and nitrogen-based fertilisers but these have frequently been correlated with the adoption of the exploitative strategy and declines in species richness (Crawley *et al.*, 2005; Maire *et al.*, 2009). The partial recovery of species richness in temperate grasslands with long-term use of heavy fertilisation was found to be a very slow process and trait composition was found to not be affected (Pierik *et al.*, 2011). Crawley *et al* (2005) found that species richness is yet to bounce back in the PGE and the results of this chapter indicate a trait composition and strategy shift to conservatism. These results contrast with those of Pierik *et al* (2010). Moreover, conservatism is often associated with low nutrient levels and unproductive systems, and therefore it seems counterintuitive for the PGE plots to adopt a conservative strategy in response to fertilisation (Wright *et al.*, 2004). The key difference is the sustained fertilisation.

Long-term fertiliser application may have alleviated competition for the limiting resources typically associated with short term exploitative plant responses. The exploitative strategy is underpinned by a species' ability to pre-empt and respond to transient increases in limiting resources, such as plant available nitrogen. Sustained nitrogen-based fertilisation renders the exploitative strategy superfluous and adoption of the conservative strategy is assumed.

The convergence/divergence patterns revealed fertilisation with ammonium sulphate as a convergent pressure. Niche occupancy and breadth at the multi-trait level, evenness and divergence of leaf thickness, leaf nitrogen content and leaf C:N all declined. The finding of convergence supports the original hypothesis of this chapter and reinforces previous research from the PGE that highlighted the negative impacts of fertilisation and ammonium sulphate application (Crawley *et al.*, 2005). The reduction in niche space occupancy and breadth suggests functional destabilisation through reduced functional redundancy. This contrasts with and could shed light on Silvertown's (1980) conclusion that with regards to plant-life form abundance and biomass, the plots of the PGE appear to be at climax community. This shows that a system can be both taxonomically stable and functionally insecure at the multi-trait level, and for singular foliar traits (leaf thickness, leaf nitrogen content and leaf C:N). This indicates that long-term fertilisation reduces a system's resiliency to cope with future pressures.

Ammonium sulphate fertilisers are thought to exacerbate the strength of the abiotic filter through decreasing the pH of the soil, which has ramifications for the mineralisable nitrogen and organic matter content. This has been linked to the absence of nitrifying bacteria and worms (Richardson, 1938). The positive impacts of liming were reported by Crawley *et al* (2005) – two extra species gained per unit of soil pH increase. The present study found that pH had a positive impact on the divergence of leaf nitrogen content and therefore has the potential to reverse the convergence observed for ammonium sulphate application. Tilman and Isbell (2015) further noted that in plots fertilised with ammonium sulphate competition for light resources is fierce and taller species dominate. This chapter found reductions in plant height at the community level with the application of ammonium sulphate. Moreover, the convergence/divergence at the single-trait level indicated specific leaf area as the locus of the biotic filter. Interspecific competition is, thus, related to differentiation in resources governed by specific leaf area. This supports the conclusion of 4.4.2. whereby partitioning in the temporal niche promotes coexistence; since specific leaf area is commonly coupled with relative growth rates and leaf life span (Chesson, 2000).

4.4.3b. Mineral Fertilisers

The response trait profile and convergence/divergence patterning in response to mineral fertilisers (particularly potassium sulphate and sodium sulphate) had contrasting effects to fertilisation with ammonium sulphate. Mineral fertilisation partially satisfied the original hypothesis in stimulating a response typical of the exploitative strategy but differed in illustrating divergence at the multi and single-trait level.

The community-weighted means of specific leaf area and leaf thickness were found to increase and decrease respectively, suggesting an exploitative strategy. Research into the effects of mineral fertilisers on functional structure and composition of temperate grassland has been limited and many inferences have been made under the assumption that responses to mineral fertilisation coincide with nitrogen-based fertilisation. This chapter found that mineral fertilisation favours the adoption of the exploitative strategy, supporting the fertilisation-exploitative relationship. Plant species adopting the exploitative strategy are thought to inhabit high-nutrient and productive environments. The adoption of the exploitative strategy could be deduced to be a rapid response (reduced community-weighted mean of seed mass) to mineral fertilisation and therefore highlights soil minerals content, particularly potassium and sodium, as limiting resources in the PGE. Results from the PGE have indicated that nitrogen, phosphorus, and potassium are all limited to the most abundant grass species and potassium was particularly limited to populations of *Taraxacum* (Tilman, 1982; Tilman *et al.*, 1999). *Taraxacum* abundance increased in response to potassium sulphate application despite being deemed a poor potassium competitor. The importance of limitations in potassium and other micronutrients has been expressed by scholars and is suggested to restrict aboveground grassland productivity (Kayser and Isselstein, 2005; Richardson *et al.*, 2009; Fay *et al.*, 2015). This chapter reinforces the importance of aboveground grassland productivity, as an increase in the community-weighted mean of plant height was associated with potassium sulphate and sodium sulphate application.

Mineral limitation can explain the functional divergence observed at the multi-trait and single-trait level. Mineral fertilisation restored the limiting resource, creating

new niche spaces. This enabled a greater range of plant species to germinate and establish, causing divergence and decreased evenness in specific leaf area, leaf thickness and leaf C:N. This suggests that mineral fertilisers have the capacity to alter the distribution and range of leaf morphology, chemistry and growth traits. This divergence in foliar traits exhibits interspecific competition for light resources and adoption of the shade avoidance strategy (Gommers *et al.*, 2013). Franklin (2008) outlined common adaptations in morphology traits; taller plants with accelerated growth rates position their leaves higher in the strata capturing a greater quantity and quality of light (red: far-red). This can be deduced from the increases in community plant height and specific leaf area.

4.4.5. The Duality of the Biotic Filter

The results from the PGE presented different responses to fertiliser application, and these convergence/divergence patterns support the theory of resource competition and limitation. However, the results of this study have, thus far, portrayed and analysed the biotic filter solely in the context of divergence, but there is evidence of convergence in the PGE in response to the distribution of grass species.

The taxonomic measures of grass species (richness, diversity, and evenness) demonstrated that in temperate grasslands, and the PGE, the strength of the biotic filter is determined by the number and distribution of grass species. The richness, diversity, and evenness of grass species were found to largely impact on the convergence/divergence at the multi- and single-trait level and have a lesser effect on the traits of dominant species (community-weighted means). This supports Lavorel and Garnier's (2002) notion of the biotic filter governing the distribution and breadth of subordinates.

The uniqueness of the results from this study lies in the isolated effects of taxonomic measures on the functional structure and composition of the PGE. Grass species' evenness decreased the utilisation and breadth of niche spaces, specifically governing convergence in leaf dry matter content and randomness in the distribution of specific leaf area, leaf thickness, leaf nitrogen content and leaf C:N.

This convergent pressure of the biotic filter was not originally hypothesised in the Response-Effect Framework but scholars are increasingly documenting instances of convergence in grassland systems (Louault *et al.*, 2005). Strong convergence in dispersal and persistence traits prevailed in more dynamic, formerly intensively managed, grasslands due to competitive exclusion and dispersal limitation (Saar *et al.*, 2017). These productive systems are likely to be dominated by superior competitors (tall and fast growing) that disproportionately exploit local resources and skews the distribution of trait values to a competitive optimum. Moreover, competitive exclusion has been noted in the PGE by Silvertown *et al* (2006) and the results of this chapter provide additional evidence for this.

Greater grass species richness and diversity was found to be indicative of divergence. Saar *et al* (2017) noted that ancient, and unimproved, grasslands showed trait patterning common to niche differentiation (divergence). Unimproved grasslands are suggested to be low productive and species-rich systems, therefore interspecific competition for local resources is likely to be high (Hejcman *et al.*, 2013). Evidence of trait divergence aligns with the traditional interpretation of the biotic filter, and for the PGE, divergence was found in plant height, seed mass, leaf dry matter content and leaf C:N. The divergence in plant height confirms the recommendation of other authors to integrate plant height as a key competitive trait, as plant height coincides with a species' competition coefficient and is closely associated with the interception of solar radiation and shade avoidance strategies (Park, Benjamin and Watkinson, 2003; Franklin, 2008; Laughlin, 2014a). The increase in the divergence of foliar traits and seed mass indicates the multitude of niche axes grass species can impact.

4.4.5. Future Directions

Traditionally, abiotic and biotic filtering have been considered as convergent and divergent forces respectively (Funk *et al.*, 2008). In recent years, scholars have revealed the flaws in the original Response-Effect Framework and suggested that false negative/positive conclusions are commonly made from observational data based on the occurrences of species (Kraft *et al.*, 2015). This chapter reinforces the arguments that abiotic and biotic filters should not be dogmatically confined to

convergence and divergence. Additionally, inferring the absence/presence of filters abstracts the complex interactions of simultaneously acting abiotic and biotic processes, which are difficult to extricate and isolate from observational data. True abiotic filtering should be inferred from investigations absent of biotic interactions and should be based on changes in species performance (growth rates, establishment, and persistence of species) across integrated environmental conditions. A more nuanced definition of abiotic filtering has been defined as "acts on the components of direct survival and reproduction and on intrinsic growth rate simultaneously, as a result, leads to shifts in the abundance and/or presence/absence of species" (Cadotte and Tucker, 2017). Cadotte and Tucker (2017) posited questions regarding the impact of phylogenetic relatedness of species and abiotic filtering, the scale dependencies of the abiotic and biotic filters, and the multiple facets of environmental pressures acting simultaneously. Lavorel and Garnier (2002) largely ignored concurrent abiotic filters and instead viewed temperate grasslands as a product of ordered hierarchies. Unfortunately, the disintegration of abiotic filters has resulted in the reductive definitions of independent, and somewhat inconsistent, plant ecological strategies. The overlap in their trait profiles is indicative of their co-dependency. Future research should address the questions considered by Cadotte and Tucker (2017) and concentrate on defining a unified ecological strategy incorporating response traits from a range of plant organs (roots), life stages and accounting for intraspecific variability. These general issues in plant functional ecology are discussed in further detail in 4.4.5a. and 4.4.5b.

4.4.5a. Expansion of Response Traits

Root response traits are often neglected and require further attention. Specific root length is analogous to specific leaf area and is suggested to be a functional unit of root biomass that responds to nutrient supply gradients (Tracy and Marino, 1989). Soil nitrogen gradients are documented to be associated with root tissue density strategies that determine individuals' and species' fitness; nitrogen enrichment impacts tissue density, lifespan, resistance to physical damage, branching, mycorrhizal infection and nitrogen use efficiency (Ryser and Lambers, 1995; Craine *et al.*, 2001; Johnson *et al.*, 2003). These phenotypic patterns are assumed to be similar along nutrient supply gradients and among species and follow the acquisitive

vs conservative strategies of leaf economics (Craine *et al.*, 2005). N-exploitative individuals/species were found to have thin roots with high nitrogen concentrations and low structural fractions (e.g. lignin), contributing to a high root respiration rate and shorter root lifespan, and greater allocation to rhizomatous growth (Kitajima and Tilman, 1996; Roumet, Urcelay and Díaz, 2006; Kembel and Cahill, 2011; Yanai, Fahey and Miller, 2013). The integration of root traits into the leaf economics spectrum, however, is contested. Craine et al (2005) found that a single, uniform and global relationship between leaf and root traits in ninety grass species from four grassland regions was unlikely, but other investigations have highlighted that root traits respond similarly to above-ground traits with respect to soil fertility gradients at both small and large scales (Kramer-Walter *et al.*, 2016). Kramer-Walter et al (2016) further suggested that not all root types complied with and resembled the treatment of root types (e.g. fine or coarse) as separate functional entities with corresponding root traits.

The differentiation of coarse and fine root types has been achieved through root diameter. Fine roots (thin; <2mm) respond to water and nutrient gradients and are responsible for resource acquisition strategies, whereas coarse roots (thick; ≥2mm) respond to nutrient gradients by storing carbohydrates and mineral nutrients and support vegetative reproduction (Kramer-Walter *et al.*, 2016). There is contrasting evidence in the responses of fine and coarse roots along nutrient and water gradients (Fitter, 2002). Kramer-Walter et al (2016) found that specific root length and root diameter were independent of tissue density strategies in response to a soil fertility gradient. It is likely that these traits vary along an environmental gradient; root diameter has been linked to precipitation gradients, where thin highly branched roots were associated with low rainfall (Li *et al.*, 2017). This suggests that root traits are multidimensional and highlights the importance of inter-root type variation.

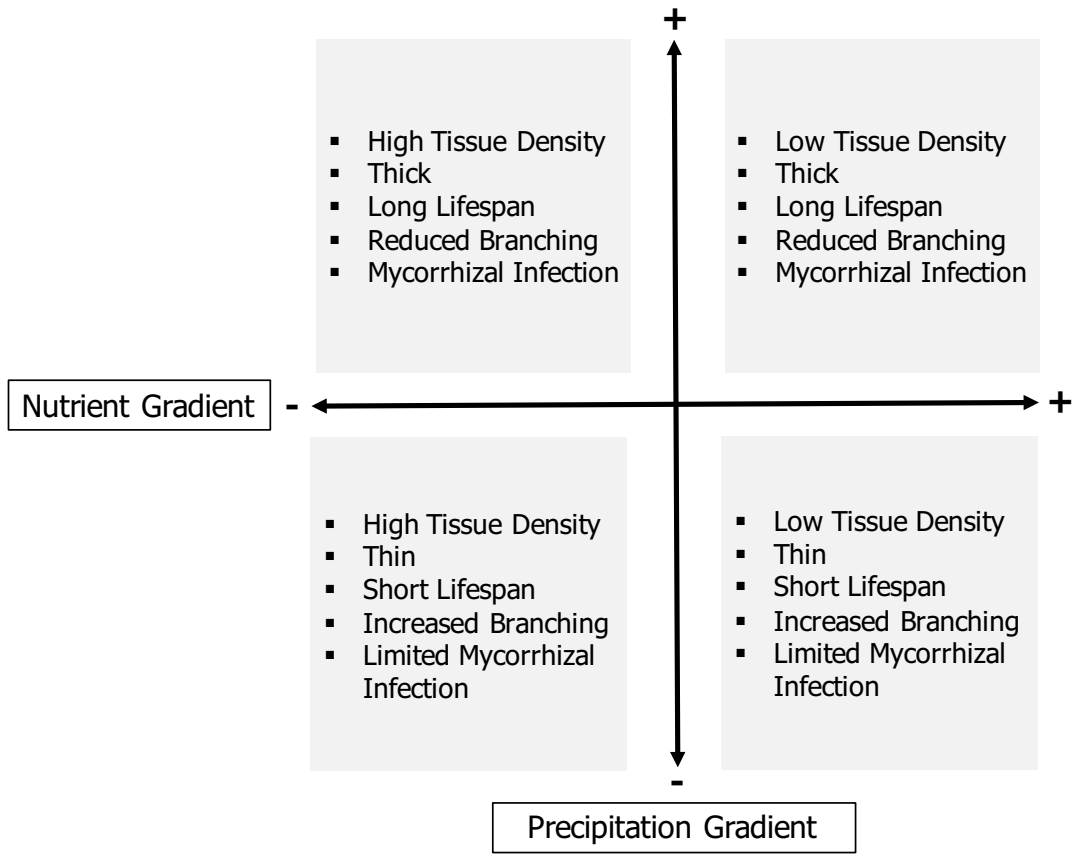

Figure-4.4.3. Graphical illustration of the multidimensional nature of root traits. Root tissue density varies along the nutrient gradient much like the leaf economic spectrum, whilst others such as root diameter, specific root length, branching intensity and mycorrhizal infection associate with different gradients, such as precipitation.

Inter-root type trait variation requires a greater understanding of the traits involved in root construction, their placement in the soil profile and their mycorrhizal colonisation, and their responses to a plethora of different environmental and management pressures. Such a high degree of complexity has left the study of root traits in its infancy, particularly in response to global change (Bardgett, Mommer and De Vries, 2014). Elevated atmospheric CO_2 was found to increase root length by 26%, diameter by 8.4%, total root biomass by 28.8%, root respiration by 58.9%, exudation by 37.9%, and mycorrhizal colonisation by 3.35% (Nie *et al.*, 2013). Elevated atmospheric CO_2, thus, was suggested to promote root ontogenesis.

Ontogenetic changes in response trait relationships and syndromes are very rarely researched despite their recognition in root and leaf economic traits (Mokany and Ash, 2008; Mason and Donovan, 2015). Leaf area, stomatal size, conductance and photosynthetic rate of *Protea* species and nitrogen content, leaf mass per area of three *Helianthus* species have been found to vary across whole-plant ontogeny (Carlson and Holsinger, 2012; Mason and Donovan, 2015). Damián et al (2017) reported the first ontogenetic changes in both the magnitude and pattern of foliar trait-trait relationships. They found that foliar trait-trait relationships were almost three-fold weaker in juvenile plants than reproductively mature individuals, suggesting that weaker correlations among traits enabled juveniles to quickly fine-tune their responses to environmental and management pressures. The ontogenetic transition between vegetative and reproductive phases, however, is thought to limit the plasticity of individual response traits, and instead, responses to environmental and management pressures are elicited through trait syndromes, such as the resource acquisition-conservation continuum (Damián *et al*., 2017). Such changes have been noted in the heteroblasty (changes in leaf size, shape and trichome density) in *Arabidopsis thaliana,* and the hormonal regulation of growth and defence traits in *Nicotiana attenuata* (Huijser and Schmid, 2011; Brütting *et al*., 2017). Furthermore, plant size/biomass was highlighted as the secondary modulator of ontogenetic changes in response trait expression and relationships; larger plants are suggested to experience greater micro-environmental heterogeneity and greater sectoriality (the isolation of plant organs), which promotes the division of the plant economic spectrum into the leaf, stem and root subsidiaries. Studying complex response trait relationships with an ontogenetic perspective provides an excellent opportunity to build a bridge between developmental biology and evolutionary and functional ecology.

4.4.5b. Intraspecific Trait Variation and Environmental/Management Pressures

Typically plant functional strategies have been defined according to interspecific responses to environmental gradients. However, the importance of intraspecific variability in responses/strategies to changing environmental conditions is gaining wider recognition. Intraspecific trait variation is defined as the ability of an individual to adapt their fitness (e.g. growth response) to disturbance and environmental heterogeneity (Valladares, Gianoli and Gómez, 2007). Intraspecific

variation is surmised to facilitate responses to environmental pressures through two mechanisms – adaptation and acclimation. Adaptation refers to the underlying variation at the genetic level between individual genotypes, which is most evident in developmental processes that determine resource allocation. Acclimation, also known as phenotypic plasticity, is the potential of each individual genotype to produce multiple phenotypes (Albert *et al.*, 2011).

Intraspecific trait responses have largely been tested in high-nutrient environments, focussing on traits associated with resource uptake (Violle *et al.*, 2009). Siefert *et al* (2014) found that intraspecific variation was most important for vegetative height response to the edaphic environment, namely available phosphorus, and least for leaf area and leaf dry matter content. Intraspecific variation in specific leaf area, leaf nitrogen content and leaf phosphorus content was found to play a strong role in driving the community-level responses to local variation in soil mineral nitrogen in the forest understorey of northern Sweden (Kumordzi, Wardle and Freschet, 2015). Leaf dry matter content is believed to be less plastic than specific leaf area; intraspecific variation in specific leaf area was found to account for 30% of variability across nutrient gradients, whereas intraspecific variation in leaf dry matter content of *Arabidopsis thaliana* was associated with temperature of the species' geographical origin (Albert *et al.*, 2010; May, Warner and Wingler, 2017). The underlying mechanism of intraspecific trait variation (adaptation or acclimation) remains untested, as does the intraspecific variation of plant traits in response to diverse environmental/management factors, especially herbivory (Reese, Ames and Wright, 2016).

Determining when and where intraspecific trait variation should be accounted for is debated. In the light of economic viability, measuring every trait for every individual from every ecosystem and in every environmental context is virtually impossible, thus understanding the relative importance of intraspecific variation for individual traits and environmental gradients is critical. The 'Spatial Variance Partitioning' Hypothesis envelops these notions assuming at the finest (organisational or spatial) scales that intraspecific trait variation is more important than interspecific differences (Albert *et al.*, 2011). Spatial scale was also highlighted by Siefert *et al*

(2014) who suggested that an increase in scale was related to the saturation of environmental factors; intraspecific trait variation should, therefore, be considered in studies examining response traits along single environmental gradients and when studying community dynamics, using distance-derived functional diversity indices (Albert *et al.*, 2011). Albert *et al.* (2011) published guidelines of when and where to account for intraspecific trait variation and recognised that the 'Spatial Variance Partitioning' Hypothesis is poorly studied at intermediate scales (e.g. regional) and that future studies should simplify analyses by considering individuals' response trait syndromes with co-varying environmental factors.

4.4.6. Future of Plant Trait-Based Management of Temperate Grasslands

The historical management of temperate grasslands was situated in the debate about intensification or extensification. The research of functional ecology and ecosystem service science has transformed these concepts into discussions of multifunctionality vs optimisation. The multifunctional management of temperate grasslands enhances or maintains forage and/or animal production whilst allowing for biodiversity conservation, nutrient retention, erosion control, carbon storage and/or other ecosystem functions (Zhang and Schwärzel, 2017). Optimisation, on the other hand, concentrates management to maximise provision of a single ecosystem service.

Within the agricultural context, these notions have transgressed into the socio-political sphere. There is a global concern about food security because the world population is projected to reach 9.2 billion by 2050 and food production needs to increase by 70-100% (Godfray *et al.*, 2010). Western scholars and policy-makers continually point to multifunctionality as an appropriate form of sustainable land management, which promises to ensure primary production, preserve natural resources and secure long-term social and economic benefits (Zhang and Schwärzel, 2017). The decisions about implementing multifunctional practices in temperate grassland management require an advanced understanding of changes in ecosystem processes. Functional ecology and ecosystem service science are immature, and challenges still remain in quantifying and evaluating ecosystem

functions; instead a leading assumption is centred on maintaining biodiversity to guarantee multifunctionality through redundancy.

Functional redundancy is grounded in the facets of functional diversity. In essence, a larger more-evenly occupied niche space encapsulates the greatest amount of functional redundancy and therefore guarantees the provision of multiple ecosystem processes and services in the face of environmental stressors (Rosenfeld, 2002). The multi-trait functional diversity indices are summarised in Figure-4.4.4 and are contextualised with reference to intensive and extensive management.

		Measure	*Intensive*	*Extensive*
The National Vegetation Classification Data	**Grazing Status**	MNND	Low Intensity	Grazed
	Farmyard Manure	MNND	Yes	No
	Improvement Status	FRic FEve MNND	Unimproved Unimproved/Improved Unimproved	Semi-Improved Semi-Improved Improved
The Park Grass Experiment	**Fertilisation/Ammonium Sulphate**	FRic FDiv	Yes Yes	No No
	Mineral Fertilisers (K and Na)	FEve FDiv	No No	Yes Yes
	Grass Species Richness and Diversity	FRic FEve FDiv	Low Low Low	High High High
	Grass Species Evenness	FEve FDiv	High High	Low Low

Figure-4.4.4. Summary of the results for the multi-trait measures from the National Vegetation Classification and the Park Grass Experiment situated according to intensive and extensive management.

As discussed in 4.4.1. and 4.4.2., the multi-trait indices highlighted illogical results from the National Vegetation Classification Data, such as the positive association of improvement status with functional diversity. It was concluded that the use of multi-trait indices distorted the analyses, and this may have been due to the spatial scale. The findings for the Park Grass Experiment provide evidence for this as the results highlighted consistencies with other scholars at the local scale, such as with fertilisation with ammonium sulphate being a convergent force (Figure-4.4.4. and 4.4.3a.) (Tilman and Isbell, 2015). The results of the PGE, thus, are of greatest

value to the multifunctional management of grasslands and suggests the avoidance of nitrogen-based fertilisation, particularly ammonium sulphate. The application of mineral fertilisers was supported, provided that the applied mineral is a limiting resource of the system. Furthermore, the PGE results have significant implications for the long-term management of temperate grasslands, as sustained improvement (over 25 years) altered the functional structure and composition to resemble an unproductive system.

Additionally, the results of the taxonomic measures of grass species suggest that taxonomic richness, diversity, and evenness can be used as surrogates to examine and manage the multifunctionality of temperate grasslands. In essence, management projects should aim to restore and maintain grass species diversity whilst reducing grass species evenness. The use of conservation or low-intensity grazing has been used across Europe as an extensive management practice to induce heterogeneity in temperate grasslands. Zhang and Schwärzel (2017) further reflected on this practice in their discussions of protection-based grazing management of temperate grasslands. They suggested low stocking rates can stimulate increased establishment and persistence of species through alleviation of interspecific competition via defoliation and creating transient niches (Muller *et al.*, 1998; Doll *et al.*, 2011). Increased coverage, thus, protects the soil surface from erosion, salinization, and desertification (Zhang and Schwärzel, 2017).

The innovative frameworks, cross-sectoral research, scientific approaches and supportive policy needed to fully implement multifunctional management is considerably lacking (Zhang and Schwärzel, 2017). This study has criticised the validity of multi-trait functional diversity indices in demonstrating coherent responses to environmental stressors. Single-trait indices are thought to expose the finer details of response traits to environmental gradients, for example with community-weighted means. Community-weighted means are commonly used as quantifiers of optimality across environmental gradients and have the capacity to inform management practices aiming to optimise the output of a single ecosystem service. The community-weighted mean of plant height is the most widely investigated plant trait in relation to green and brown biomass production, and

literature has continually validated its role in prediction (Laliberté and Tylianakis, 2012; Price and Casler, 2014; da Silveira Pontes *et al.*, 2015). Therefore, grassland management would aim to optimise community level plant height through conducive environmental and management factors; this chapter postulates low intensity grazing, semi-improvement and/or the application of mineral fertilisers (potassium sulphate and sodium sulphate) are likely to stimulate plant height responses favourable for biomass production.

Optimisation offers a more digestible interpretation of sustainable land management for grassland managers, farmers, and policy-makers. However, like multifunctionality, empirical evidence of the relationship between community-weighted means and individual ecosystem services is embryonic and its implementation has largely been unexplored. One envisions a landscape of engineered temperate grasslands designed to optimise the delivery of single ecosystem services, which are integrated into implementation frameworks at the regional and national level to promote landscape connectivity and deter fragmentation.

Trait-Based Approach to Examine the Provision of Biomass and Animal Production from Temperate Grasslands

5.1. Introduction

The study of temperate grasslands has been central to the United Kingdom's reputation in ecological research. Temperate grasslands have supplemented our understanding of core ecological concepts, such as ecological stability, plant ecological strategies, and the biodiversity-ecosystem service relationship (Hector, 1999; Silvertown *et al.*, 2006; Grime, Hodgson and Hunt, 2007). Research into the latter has primarily centred on boosting provisioning services, namely animal production (meat, dairy, wool etc.), through supporting diversity via extensification management of the biocultural landscape of the United Kingdom. Currently, temperate grasslands exist as remnants of traditional farming and are products of thousands of years of human management that have been devastated by decades of human expansion and improvement activities (heavy fertiliser application and resowing) to provide grazing and fodder for animal production (Bullock *et al.*, 2011). Extensive management has been tasked with enhancing biodiversity under the assumption that the supply of ecosystem services will be restored.

The major ecosystem services provided by temperate grasslands predominantly relate to provisioning and cultural services but European scholars have attended mostly to regulating services (Hevia *et al.*, 2017). Regulating service investigations have concentrated on agriculturally relevant services; climate regulation, greenhouse gases emissions, and pollination. The United Kingdom's temperate grasslands are suggested to sequester carbon at a rate of 242$\pm$1990kg/ha/yr, which is a higher rate than for slowly growing forests and arable land, but the impacts of grassland management on greenhouse gases and climate regulation is in its infancy (Janssens *et al.*, 2005). The intensity of management practices (intensive vs extensive) is correlated with increased methane and nitrous oxide emissions as a result of high stocking and fertilisation rates respectively, but there are few documented and consistent effects on carbon sequestration (Soussana *et al.*, 2004;

Hopkins *et al.*, 2009; Bullock *et al.*, 2011). Temperate grasslands are surmised to switch from a carbon sink to a carbon source with increasing management intensity (Liebig *et al.*, 2010).

The spill-over of pollinators from temperate grasslands is another key regulating service. Decreasing wild bee diversity, especially bumblebees, has been attributed to the diminishing abundance and range of the core foraging plants of British populations. The intensification of management of temperate grasslands has severely impacted populations of *Centaurea nigra, Lathyrus pratensis, Leucanthemum vulgare, Lotus corniculatus* and *Trifolium pratense* which are…… (Goulson *et al.*, 2005; Carvell *et al.*, 2006).

The cultural service of providing habitat for species of conservation interest was recognised in the UK Biodiversity Action Plan (UK BAP, 1992-2012). It focussed on four broad temperate grassland types (acid grassland, calcareous grassland, neutral grassland and improved grassland) to be restored or recreated to support fungi, lichens, bryophytes, vascular plants, invertebrates, amphibians, reptiles, birds and mammals (Bullock *et al.*, 2011). Other cultural services have centred on churchyards and National Parks for recreation, greenspace, education, physical and psychological health and religion, for example, churchyards are thought to be symbols of the Christian gospel of life and death (Swanwick, Dunnett and Woolley, 2003). Additionally, one of the 222 English National Parks, South Downs, support 5200 jobs and yields £177 million per annum (Barton and Pretty, 2010).

The genetic resources of temperate grasslands are valued as a provisioning service that is central to the management, restoration, and creation of species-rich grasslands. Conservation initiatives utilise a significant amount of seed sourced from temperate grasslands together with rare or traditional livestock breeds to manage temperate grasslands less intensively (Bullock *et al.*, 2011). Decreasing intensification has been linked with the increased provision of water in terms of quantity and quality; reduced stocking and fertilisation rates increase soil infiltration and decrease runoff and contamination from fertilisers, manure and slurry, which

reduces the risk of flooding and recharges aquifers (Weatherhead and Howden, 2009). Despite this, more research is needed to understand how temperate grasslands provide water. The most well-studied provisioning service is food/animal production that is typically reduced to a straightforward quantity measure of dry matter yield of cut hay (Tallowin and Jefferson, 1999). Inferences linked to the concepts of palatability and digestibility are assumed for the quality of forage and hay that is surmised to represent the quality of livestock production, but our understanding of the mechanisms underpinning the delivery of many ecosystem services and goods from temperate grasslands is lacking.

Ecosystem Service Category	*Ecosystem Service*	*Description*	*Effects of Temperate Grasslands*	*Quantity of Livestock Production*	*Quality of Livestock Production*
Supporting Services	Supporting Habitats	Habitats for breeding, reproduction, nursery, refuges and corridors.	↑*	-	+
	Soil Formation	Includes chemical weathering of rocks and the transportation and accumulation of inorganic and organic matter.	↑*	-	+
Regulating Services	Gas Regulation	Relates to biogeochemical processes including greenhouse gases, photochemical smog and volatile organic compounds.	↓	+	-
	Climate Regulation	Atmospheric processes and weather patterns.	↗	±	±
	Water Regulation	Spatial and temporal distribution of water through atmosphere, aquifers, rivers, lakes and wetlands.	↗	-	-
	Soil Retention	Minimising soil loss through vegetative cover, root biomass and soil biota.	↑*	-	+
	Nutrient Regulation	Transport, storage and recycling of nutrients.	↑	-	+
	Pollination	Interaction between plant and abiotic/biotic vectors in the movement of male gametes for plant production.	↑	-	+
Provisioning Services	Food	Production of photosynthetic biomass and secondary production.	↗	+	-
	Raw Material	Biomass used for other purposes than food.	↗	-	±
	Water Supply	Providing water through sediment trapping, infiltration, dissolution, precipitation and diffusion.	↗	-	±
	Genetic Resources	Self-maintenance of diversity at the species, molecular and sub-molecular levels.	↗	-	+
Cultural Services			↗	-	+

Figure-5.1.1. A summary of the effects on temperate grasslands on a range of ecosystem services and the suggested direct relationships between the different

facets of animal production and other major ecosystem services. Direction of the arrow denotes the direction and strength of impact; starred arrow indicates strongest impacts. Abbreviations: +, Positive; -, Negative; ±, Positive or Negative.

The importance of ecosystem services has gained recognition at the global and national scale. The Intergovernmental Panel on Biodiversity and Ecosystem Services is an international collaboration of decision-makers who assess the knowledge and research on the biodiversity-ecosystem services relationship to sustain nature and human wellbeing in a changing world (Schmeller and Bridgewater, 2016). Studies into this relationship are entrenched in an array of disciplines varying in their metrics of biodiversity and ecosystem services, their analytical approaches and a wide range of scales dependent on the specific management issues. Biodiversity in research has taken many forms: genotypes, species abundance and richness, populations, species functional groups, functional structure and composition indices and community/habitat area (Díaz, S. Lavorel, *et al.*, 2007; Duncan, Thompson and Pettorelli, 2015). Harrison *et al* (2014) concluded that the biodiversity-ecosystem service relationship is a system of intricate, complex and uncertain linkages. For these reasons, empirical investigations typically show inconsistent results and few are tested empirically, thus misleading scientific syntheses and management interventions (Ricketts *et al.*, 2016). Despite this, Functional Ecology is still considered the Holy Grail to improve our understanding of how biodiversity responds to environmental and management changes and the subsequent biodiversity effects on ecosystem services.

5.1.1. Plant Functional Traits and Ecosystem Services

Functional ecology, via plant functional traits, attempts to mechanistically link environmental and management drivers with ecosystem services (Figure-5.1.3) (Ricketts *et al.*, 2016). With growing recognition, research has focussed on categorising co-varying plant traits into axes of specialisation or ecological strategies, which were initially highlighted as response traits to environmental and management factors but have gained support for their effects on ecosystem properties and functions that underpin ecosystem services. The Leaf Economics Spectrum (LES) segregates exploitative and conservative plant species based on

their trait profiles, which scale-up to effects on temperate grassland nitrogen and carbon cycling, primary production, litter decomposition, soil water retention, and agronomic and cultural value (Gross, Suding and Lavorel, 2007; Lavorel and Grigulis, 2012; Grigulis *et al.*, 2013). Exploitative plants are associated with faster nutrient turnover because their protein-rich leaves have short lifespans, which senesce and induce fast litter decomposition by the soil biota. Conservative plants, on the other hand, are suggested to support carbon sequestration and soil water retention through maintaining consistent standing biomass that invests carbon and nutrients in hardy foliar structures that lose water more slowly through the growing season (Gross, Suding and Lavorel, 2007; Lavorel and Grigulis, 2012; Grigulis *et al.*, 2013). However, the use of the ecological strategies has shown inconsistent relationships with ecosystem processes/services in temperate grasslands and rangelands, but they have highlighted plant traits of significant interest, such as plant height, seed mass, leaf nitrogen content, and specific leaf area.

Ecosystem Service	*Ecosystem Process*		*Impact of Leaf Economics Spectrum*	
			Conservative	Exploitati
Nutrient Regulation	Carbon Cycling	Inputs	⊖	⊕
		Decomposition	⊖	⊕
	Nutrient Cycling		⊖	⊕
Food/Raw Material	Primary Production		⊖	⊕
	Livestock Production		⊖	⊕
Water Regulation/Supply	Soil Water Retention		⊕	⊖

Figure-5.1.2. A summary of the impacts of the conservative and exploitative strategies on the supply of ecosystem processes and services from temperate grasslands. Abbreviations: +, Positive; -, Negative.

The functional structure and composition of temperate grasslands have recently been at the forefront of understanding the provision of ecosystem services. The structure and composition of a plant community has been measured by a multitude of indices, which represent two leading schools of thought in how plant effect traits

support ecosystem services. The Biomass-Ratio Hypothesis surmises that the delivery of ecosystem goods and services is driven by the traits of the most abundant species in the community, and is calculated through average trait value per unit biomass (community-weighted means, CWM) (Grime, 1998; Violle *et al.*, 2007b). Plant community effect traits on ecosystem services have been primarily attributed to CWMs and are suggested to be a stronger determinant than measures of the Functional Diversity Hypothesis (Laliberté and Tylianakis, 2012).

The Functional Diversity Hypothesis has been deconstructed into three different, but complementary, concepts of richness, evenness, and divergence that can be measured at the single or multi-trait level (Mouchet *et al.*, 2010). Functional diversity effects on ecosystem services are underpinned by the insurance hypothesis that assumes high functional diversity provides a greater guarantee of the maintenance of ecosystem services through functional redundancy (Yachi and Loreau, 1999). Greater occupancy (functional richness, FRic), an even distribution (functional evenness, FEve) and breadth (functional divergence, FDiv) of the niche space, therefore, possess a greater probability of functional redundancy in the community (Villéger, Mason and Mouillot, 2008). In this way, functional diversity contributes to the sustainability of temperate grassland systems through increased resilience and predictability of ecosystem service outputs (Pakeman, 2014b). Functional diversity has been shown to support a variety of ecosystem processes and services, such as fodder and wood provision, carbon sequestration, soil nutrient retention and pollination (Díaz, S. Lavorel, *et al.*, 2007). Functional diversity can be represented at the single-trait or multi-trait level. Single trait indices are assumed to provide a greater complexity of trait effects on ecosystem services, however, multi-trait indices have been found useful in predicting the delivery of multiple processes and services (Cornwell and Ackerly, 2009; Mouillot *et al.*, 2011; Butterfield and Suding, 2013).

In comparing the two hypotheses, evidence for the Biomass Ratio Hypothesis is paramount, however, scholars are indicating that neither hypothesis sufficiently predicts provision of ecosystem services. Combining these hypotheses and integrating single-trait and multi-trait indices are important for future studies (Funk

et al., 2016). Additionally, analysing the relative contributions of environmental and management pressures and plant trait hypotheses on the provision of ecosystem services is still in question. Figure-5.1.3. demonstrates four different models of varying complexity that could explain the provision of ecosystem services, ranging from the most reductive (environmental and management pressures alone, Model-1) to the most complex (environmental and management pressures + biomass ratio hypothesis + functional diversity hypothesis).

The upcoming sections review the literature and the evidence for each of the models outlined in Figure-5.1.3. in the context of food production, specifically livestock production from temperate grasslands. Livestock production is presented as two different processes; quantity of forage represented by green and brown biomass (hay), and quality of forage through the concepts of palatability and digestibility. This separation is due to the lack of research into sophisticated measures of food production from grasslands, such as yield and quality of meat, milk, and wool.

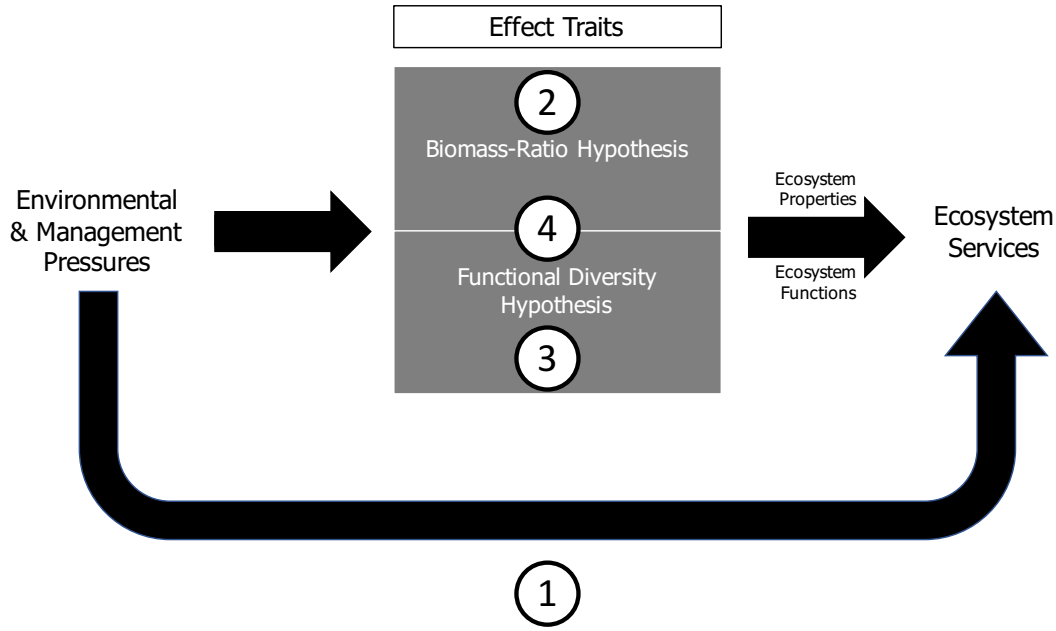

Figure-5.1.3. Conceptual models of the Response-Effect framework for the provisioning of ecosystem services. Four models are proposed: 1) environmental

and management pressures alone; 2) environmental and management pressures plus community-weighted mean(s); 3) environmental and management pressures plus functional diversity indices; 4) environmental and management pressures and combination of community-weighted mean(s) and functional diversity indices.

5.1.2. Quantity of Forage Material (Green and Brown Biomass)

Measures of green and brown biomass have focussed on dry matter yield (Tallowin and Jefferson, 1999). Early research centred on the functional separation of grass and legume species, and the general consensus is that forage legumes determine green biomass yield but are not appropriate for the production of brown biomass as they have thick stems which dry slowly and easily lose leafy material when handled (Rihawi *et al.*, 1987; Gierus *et al.*, 2012). Plant traits associated with high biomass production have been related to the size (plant height, leaf width, and stem size) and the Leaf Economics Spectrum. Genotypes conferring plant height, leaf width, and stem size were found to have a positive effect on biomass yield (Price and Casler, 2014). Foliar traits correlated to relative growth rate have also been highlighted (Cingolani, Posse and Collantes, 2005). The mechanism by which these effect traits underpin the delivery of green and brown biomass is debated, but plant height is widely recognised as the leading determinant.

The community-weighted mean (Model 2) of plant height has been widely implemented in green and brown biomass production research and sufficiently explained biomass quantity, as well as its inter-annual variation (da Silveira Pontes *et al.*, 2015). The positive effects of dominant tall species on aboveground, and belowground, biomass was also found by Laliberté and Tylianakis (2012) for grasslands at the global scale. Aboveground biomass has been found to be best explained by CWMs and not functional diversity indices; specifically seed mass, specific leaf area and leaf nitrogen content have been highlighted as positive influences and leaf dry matter content a negative one (Garnier *et al.*, 2004; Duru *et al.*, 2010; Roscher *et al.*, 2013). Studies using functional diversity indices (Model 3) have provided further evidence for the relevance of single-trait - plant height, seed mass and measures of tissue density and quality (leaf dry matter content and leaf C:N) (Garnier and Navas, 2012; Grigulis *et al.*, 2013). However, the effects of

functional diversity are highly contested and considered secondary to the Biomass Ratio Hypothesis. Grigulis *et al* (2013) reported a negative effect on aboveground and belowground production, yet divergence (measured by Rao's Q) in canopy height and seed mass was found to have positive impacts on green biomass (Kelemen *et al.*, 2015). Furthermore, the best models for predicting aboveground biomass incorporated multi-trait indices; Clark *et al* (2012) suggested the use of complementary multi-trait indices – FRic, Rao's Q and either FDis or FEve. The combined and relative effects of CWMs and single- and multi-trait functional diversity indices on biomass production is still ambiguous and greatly understudied.

Whether biomass production can be reduced to environmental and management factors, irrespective of functional and taxonomic measures, is debatable. Fertiliser application has been integral to the intensive management of temperate grasslands to increase total annual production and brown biomass yield and has been empirically demonstrated by numerous scholars (Tallowin, 1996; Kahmen and Poschlod, 2004; Schumacher and Roscher, 2009). Moreover, Tallowin and Jefferson (1999) found the addition of inorganic fertilisers on temperate grasslands increased dry matter yields by 10 to 12 tonnes per hectare per year. Environmental and management factors are commonly considered auxiliary in models that predict green and biomass production, and studies integrating these with measures of the Biomass Ratio Hypothesis and the Functional Diversity Hypothesis are inadequate. Further analyses are needed to identify the best predictive model from Figure-5.1.3. and what plant traits and indices are the strongest explanatory variables.

5.1.3. Quality of Forage Material

There is growing recognition that the taxonomic, and thus functional, structure and composition of temperate grasslands has the capacity to control the quality of livestock outputs, such as the nutritional value, taste, appearance, and smell of meat and dairy products (Bullock *et al.*, 2011). Despite this, plant trait-based research has concentrated on forage quality as defined by the traits assumed to govern palatability and digestibility. Early research pointed to herbage maturity as the dominant factor controlling forage quality, with more robust studies confirming the fundamental plant traits that dictate palatability and digestibility (Buxton, 1996).

Palatability is managed by plant physiology and non-structural carbohydrates and mineral components, whereas digestibility is governed by the composition of structural carbohydrates.

5.1.3a. Palatability of Forage Material

Palatable forage is associated with plant traits derived from morphology and non-structural carbohydrates and minerals. The simplest measures are those that infer physical defences against herbivory, such as thorns, spines, hooks, rough leaf margins, high tensile leaves, and have been shown to drive short-term forage selection in goats and sheep (Lambert, Jung and Costall, 1989; Díaz, Noy-Meir and Cabido, 2001; Mkhize *et al.*, 2014). Furthermore, a fall in the voluntary intake of triticale-vetch hay was associated with the development of awns of triticale as green biomass matured (Rihawi *et al.*, 1987). Maturity was also a factor reported by Mkhize *et al* (2014) in foraging behaviour, as Nguni goats preferred new leaves on new shoots due to the allocation of crude protein in foliar structures.

Crude protein concentration (6.25 x leaf nitrogen content) has been a core component that drives leaf/forage quality and herbivore selectivity. Decreases in crude protein were found to reduce the palatability of brown alfalfa (Fonnesbeck *et al.*, 1986; Tallowin and Jefferson, 1999; Cingolani, Posse and Collantes, 2005). The importance of tissue nitrogen content was further stressed by Lavorel and Grigulis (2012) who concluded that nitrogen content controls mountain grassland biochemistry. Leaves were found to have twice as much protein and higher concentrations of minerals, such as calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), silica (Si) and sodium (Na) than stems (Tallowin and Jefferson, 1999). Lambert, Jung and Costall (1989) found an increase in foraging associated with P content, ranging between 1-1.8g/kg of dry matter, and low Si content; silica was found to function as a chemical defense against herbivory (Adler *et al.*, 2004).

Despite the plethora of evidence highlighting plant traits of palatability, research into the models outlined in Figure-5.1.3 have been limited. Deducing from the literature, key environmental parameters that regulate palatability (namely leaf

nitrogen content) were found to be altitude, soil fertility status (nitrogen) and soil water content (Buxton, 1996; Lavorel and Grigulis, 2012).

Buxton (1996) found nitrogen fertilisation increased the crude protein of grasses and water stress was associated with the reallocation of soluble carbohydrates from foliar to structural organs (stems and roots). However, the impact(s) of environmental and management factors and palatable plant traits have on measures of livestock production is minimal. Growth and body condition of livestock was, however, found to be dictated by forage protein and mineral content and was higher in hay yields from improved grasslands (Tallowin and Jefferson, 1999).

5.1.3b. Digestibility of Forage Material

Digestibility is commonly affiliated with the amount and composition of structural carbohydrates. Investment in stems was been found to decrease the digestibility of forage material through increased structural carbohydrates (cellulose, hemicellulose, and fibre) and lignification (Gierus *et al.*, 2012; Gardarin *et al.*, 2014). Buxton (1996) found ruminants have the ability to metabolise less than one-third of their energy. The remaining two-thirds of structural carbohydrates decrease the rate of forage movement in the gut passage that leads to curtailed ingestion and a reduction in the efficiency of digestion and thus decreases the calorific value of forage material – the bulk hypothesis (PENNING *et al.*, 1994). The plant cell walls amass the energy-rich cellular non-structural carbohydrates (Fonnesbeck *et al.*, 1986).

Fibre content and leaf dry matter content have been the prominent traits employed to exemplify forage digestibility. Fibre content is comprised of three exclusive fractions; neutral detergent fibre (cell walls) and acid detergent fibre (cellulose, hemi-cellulose and lignin) (Van Soest and Wine, 1967). NDF concentrations in four cool-season grasses were found to be higher in stems than leaves and were subject to increases with plant maturity (Buxton, 1996). Ansquer *et al* (2009) found that leaf dry matter content was positively correlated with fibre content and was a good predictor of plant digestibility. The significance of leaf dry matter content has been

echoed by Gardarin *et al* (2014) and Pakeman (2014). Leaf dry matter content had the most consistent (negative) effects on digestibility; specific leaf area and leaf nitrogen content had positive impacts (Gardarin *et al.*, 2014).

Much like palatability, the influences of environmental and management factors have been deduced from research on improved and semi-improved grasslands. Tallowin and Jefferson (1999) found that indigestible constituents were lower in brown biomass yielded from improved grasslands and decreased from 70-50% over the growing season (April to September). The significance of improvement status was reiterated by Lavorel and Grigulis (2012) together with altitude. However, the temperature rise was found to decrease digestibility of green biomass through faster maturation that leads to higher NDF concentrations (Buxton, 1996). The relative contributions of environmental and management factors and the Biomass Ratio and Functional Diversity hypotheses of digestibility-related traits was investigated by Pakeman (2014), who found the community-weighted mean of leaf dry matter content was a robust, but relatively weak predictor of livestock production, and was present after accounting for rainfall, however, functional diversity indices were not helpful in explaining livestock production from temperate grasslands. At present, the role of leaf dry matter content has been widely appreciated and identified as the key linkage between functional structure and composition, via dominant species/traits, and animal production.

5.1.4. Quantity and Quality of Livestock Production

Plant trait-based research on the outputs of livestock production has been severely limited. Instead, scholars have focussed on defining response traits to herbivory and categorising plant species' strategies (tolerance vs avoidance) rather than quantifying the impact of effect traits on the outputs livestock production. Research has stressed the importance of management effects on grazing pastures with particular impacts relating to stocking rates (livestock units per hectare per year), the rate, quantity, and quality of meat and cheese production.

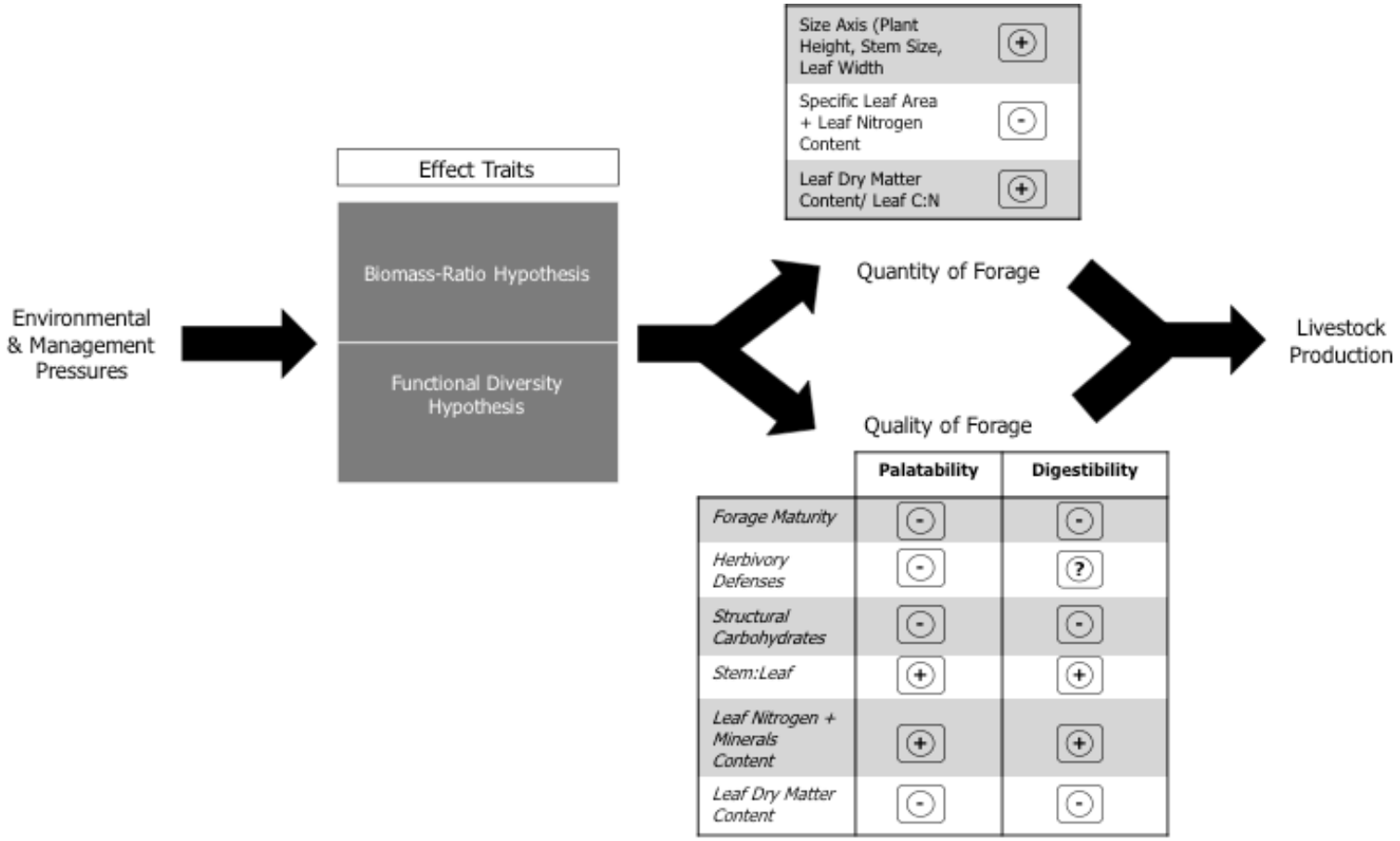

Figure-5.1.4. Conceptual summary of livestock production. The effect trait hypotheses scale-up to the measures of forage quantity and quality. Forage quality is sub-defined into the notions of palatability and digestibility. The known relationships with plant functional traits are illustrated. Abbreviations: +, Positive; -, Negative; ?, Unknown .

Improved grasslands (e.g. heavy fertiliser application) were found to support over three times the stocking rate of livestock as unimproved and semi-improved grasslands, and nitrogen fertilisation during the grazing period showed significant increases, by a factor of four, in the rate of live weight gain (Tallowin, 1996; Tallowin *et al.*, 2005; Fraser *et al.*, 2009). Live weight gain was also recorded to be the highest from sown improved leys of ryegrass and legumes, which are suggested to be the best fattening pastures (Hopkins and Wilkins, 2006). Fraser *et al* (2009) further reported a 15% increase in final carcass weight on improved pastures.

The quality of livestock products has also been linked to grassland improvement; lamb meat originating from semi-improved grasslands was found to taste and smell worse and had higher amounts of polyunsaturated fats than that reared on unimproved pastures (Bullock *et al.*, 2011). Additionally, cheese derived from improved grasslands was found to be more bitter and rancid in odour than its semi-improved counterpart (Coulon *et al.*, 2004). Coulon *et al* (2004) inferred that the botanical structure and composition of these grasslands indirectly affected the milk proteins, fats and enzymes and the diversity of microbes through the transferral of species-specific chemicals, such as terpenes and carotenes. These biochemical traits highlight a potential gateway to the study of livestock production. However, the relative contributions of environmental and management factors, indices of the Biomass Ratio and Functional Diversity Hypotheses and the quantity of forage are ill-defined. Pakeman (2014) is, thus far, the only investigation to account for the collective role of environmental factors and plant traits on livestock production (rainfall and community-weighted mean of leaf dry matter content). However, his measure of livestock production (livestock units/ha/yr to achieve a set vegetation height) can be criticised for being too simplistic and there is a need for more sophisticated measures that represent the rate, quantity, and quality of livestock products.

5.1.5. Scope of the Chapter

Scholars have noted the inadequacy of the Functional Diversity Hypothesis and there is much empirical support for the use of community-weighted means. Therefore, the general hypothesis for this chapter is that the Biomass-Ratio

Hypothesis is likely to play a greater role in supporting the provision of ecosystem services from temperate grasslands than the Functional Diversity Hypothesis. The chapter further proposes and tests four specific hypotheses to understand the provision of biomass and livestock production from temperate grasslands using the general models of Figure-5.1.3.

5.1.5a. Hypothesis 1: Quantity of Forage Material

Temperate grasslands have long been managed and improved, predominantly with inorganic fertilisers, to increase green biomass and the yields of brown biomass. The combination of inorganic nitrogen fertilisation and dominant species possessing traits related to the Size Axis (plant height and seed mass) will provide the best explanatory power and will demonstrate the significance of Model 2 of Figure-5.1.3.

5.1.5b. Hypothesis 2: Quality of Forage Material

Quality of forage material has been inferred from structural and leaf chemistry traits that are thought to determine forage palatability and digestibility. Dominant species with palatable and digestible traits (high leaf nitrogen content, low leaf dry matter content and leaf C:N) will underpin the quality of forage material, and climate and environmental factors will play an auxiliary role (Model 2 of Figure-5.1.3).

5.1.5c. Hypothesis: 3: Quantity of Livestock Outputs

Quantity of livestock production was studied by Pakeman (2014) and was operationalised by the number of livestock units per hectare per year to achieve a set vegetation height. Climate and environmental variables and dominant species with palatable and digestible traits, predominantly leaf dry matter content, will be significantly and positively related to the quantity of livestock outputs (Model 2 of Figure-5.1.3).

5.1.5d. Hypothesis 4: Quality of Livestock Outputs

The incorporation of dietary compounds into livestock products (meat, milk and cheese) has been documented and the effects on consumers' opinions, preferences and spending has been postulated. Satisfying the market is essential for livestock producers and adjusting feed regimes is common to ensure animals finish with the highest quality products and the stock are brought to market at the best time. Compounds associated with nutritional value, such as protein content (leaf nitrogen content X 6.25), will be a key variable in predicting the quality of livestock products and, given the evidence that shows the positive impact of plant-available soil nitrogen on leaf nitrogen content, soil nitrogen will also play a positive role.

The aim of this chapter was to shed light on the mechanisms that support the provision of ecosystem services from temperate grasslands. Using biomass and food production as examples, this should increase the understanding of functional ecology generally, and inform the future management of livestock production systems through insights into the effects of environmental variables on the quantity and quality of both forage and animal products via the medium of plant effect traits. Increased knowledge allows farmers and political bodies to make justified decisions on whether intensive or extensive management of temperate grasslands should be pursued.

5.2. Statistical Methods

5.2.1. Dimensionality Reduction through Principal Components Analysis of the North Wyke Farm Platform Data

A principal component analysis (PCA), based on a correlation matrix, was conducted to reduce the dimensionality of the climate and environmental variables, the herbage parameters and the measures obtained from the animal data as accessed via the North Wyke Farm Platform data portal. Matrices, as outlined in Table-5.2.1, were constructed, standardised and submitted for PCA. The number of dimensions to be retained was based on coordinates (<0.2) on the main PCA axes and the number of components needed to explain total variance was between 70-80%.

5.2.2. Testing the Mechanisms of Forage Material Production from the North Wyke Farm Platform and the Park Grass Experiment

Best subsets regression was employed to identify the best predictors of 1st cut dry matter yields from seventy-plots of the PGE and the average sward height, total carbon and nitrogen of dry matter from the NWFP. Each of these dependent variables was analysed separately. A total of fifteen model classes were tested, representing the one-way, two-way, three-way combinations and full model classes. The full model included: environmental descriptors, community-weighted means, single-trait functional diversity indices and multi-trait functional diversity indices. The R package *leaps* (function *regsubset*) was used to search for the best model, using the leaps and bounds algorithm, at each level of complexity (number of variables) for the fifteen classes. Values of the models Bayesian Information Criterion (BIC) were used to select the best models at each level of complexity and the best model for each dependent variable. The adjusted R^2 and F-statistic were calculated to examine statistical significance. The percentage change in BIC of adding each variable class (climate and environmental, Biomass-Ratio Hypothesis, Functional Diversity (Single) and Functional Diversity (Multi)) was calculated to cross-examine the degree of improvement/deterioration with each variable class addition.

The climate and environmental data for the North Wyke Farm Platform was represented as mean annual and range values due to the mismatch of the 15-minute data and the sample identifiers (SP_ID) of the botanical and herbage surveys.

5.2.3. Examining the Mechanisms of Livestock, Lamb and Cattle Production from the North Wyke Farm Platform

The measures of livestock production (rate of weight gain, final live weight, and dead weight value) for cattle and lamb were analysed separately, and in combination, to reveal the underlying relationships of climate and environmental variables, community-weighted means, single- and multi-trait functional diversity indices, and the measures of green biomass (average sward height) and quality (total C and N of dry matter) with them.

<table>
<tr><th rowspan="2">Climate and Environmental Variables</th><th rowspan="2">Herbage Parameters</th><th colspan="2">Animal Data</th></tr>
<tr><td>Cattle Data</td><td>Lamb Data</td></tr>
<tr><td>Ammonium/Ammonia (mg/l)
Nitrite & Nitrate (mg/l)
Soil pH
Precipitation (mm)
Soil Moisture at 10cm (%)
Soil Moisture at 20cm (%)
Soil Moisture at 30cm (%)
Soil Temperature (°C)</td><td>Average Sward Height (cm)
Total Carbon of Dry Matter (%)
Total Nitrogen of Dry Matter (%)</td><td colspan="2">Rate of Weight Gain (kg/day)
Abattoir Premiums/Penalties (pence/kg)
Final Live Weight (kg/day)
Cold Carcass Weight (kg)
Dead Weight Value (pence/kg)</td></tr>
</table>

Matrices		
Catchment X Climate and Environmental Variables	SP_ID X Herbage Parameters	Official Tag (Livestock Identifier) X Livestock Production Measures

Table-5.2.1. Details of the variables structure and associated data.

Cattle and lamb data were recorded per individual animal and assigned to a specific farmlet (red, blue or green), therefore issues of matching across datasets arose. The climate and environmental variables, the community-weighted means, the single- and multi-trait functional diversity indices, and the measures of forage quantity and quality were fixed at the farmlet level – mean values were calculated per farmlet. The range was also calculated for the climate and environmental variables.

The rate of weight gain, abattoir premium/penalties, final live weight, cold carcass weight and dead weight value were standardised separately for cattle and lambs. These standardised values were then combined to produce the dependent variables: rate of livestock weight gain, livestock final live weight and dead weight value. Table 5.2.1. provides a summary of all the dependent and independent variables used to analyse livestock production in the NWFP.

Livestock units (total, cattle, lamb, and sheep) were calculated according to the European Commission standard protocol (Chapter-Two). These were calculated at the catchment level and matched with climate and environmental variables, community-weighted means, single- and multi-trait functional diversity indices, and the parameters of quantity and quality of forage; all fixed (mean values) per catchment.

Least absolute shrinkage and selection operator (Lasso) regression was conducted to perform the variable selection procedure to investigate the effects of the independent variables in Table-5.2.2 on the measures of total livestock production,

cattle production, and lamb production. Lasso is suggested to produce robust and easily interpretable models and retains the good features of both subset regression and ridge regression (Tibshirani, 1996). Lasso regression was chosen as it is particularly useful when the number of observations is less than the number of predictor variables, as was found with recordings of livestock units, where the largest number of observations was eleven (total livestock units). Lasso regression was performed on model classes for each measure of cattle, lamb and total livestock.

A lasso regression, executed through the R function *glmnet*, was conducted. The *glmnet* function returns a sequence of models that can be selected using cross-validation. A 10-fold cross-validation, using the *cv.glmnet*, was performed and indicated the best model that minimised the mean cross-validated error.

<table>
<tr><th>Dependent Variables</th><th>Independent Variables (fixed per farmlet)</th></tr>
<tr><td>Cattle Production

Rate of weight gain, abattoir premiums/penalties, final live weight, cold carcass weight, dead weight value and cattle livestock units</td><td rowspan="3">Climate and Environmental Variables (Means and Ranges)

Precipitation, soil temperature, pH, nitrite and nitrate and ammonia and ammonium.

Biomass-Ratio Hypothesis

Community-weighted mean per trait

Functional Diversity Hypothesis (Single)

FRO, MNND and Range per trait</td></tr>
<tr><td>Lamb Production

Rate of weight gain, abattoir premiums/penalties, final live weight, cold carcass weight, dead weight value and cattle livestock units</td></tr>
<tr><td>Total Livestock Production

Rate of weight gain, abattoir premiums/penalties, final live weight,</td></tr>
</table>

cold carcass weight, dead weight value and cattle livestock units	*Functional Diversity Hypothesis (Multi)* FRic, FEve, FDiv, MNND and βRao's Q. *Forage Quantity and Quality* Average sward height, total C of dry matter and total N of dry matter.

Table-5.2.2: Full summary of the dependent and independent variables submitted for lasso regression. Abbreviations: FRic, Functional Richness; FEve, Functional Evenness; FDiv, Functional Divergence; MNND, Mean Nearest Neighbour Distance; FRO, Functional Regularity.

5.3. Results

5.3.1. Principal Components Analysis of the North Wyke Farm Platform

A principal components analysis was employed to explore and reduce the dimensionality of the North Wyke Farm Platform data. The variables retained for further analysis were scrutinised based on having an absolute loading of less than 0.2 across a defined number of components that explained between 70-80% of the total variation. Pearson's correlation coefficients were also calculated to further examine redundancy and reduce the number of variables to be retained.

5.3.1a. Climate and Environmental Variables

The eigenvalues indicated that the first four components explained a total of 76.593% (Figure-5.3.2). The absolute loadings demonstrated that all seven climate and environmental variables should be retained for future analyses from the principal component analysis. However, strong significant correlations (above 0.5) were found for soil temperature, soil moisture at 10cm, 20cm and 30cm (Table-5.3.1). Soil temperature was retained to reduce multicollinearity in subsequent regression, therefore there was a reduction in climate and environmental variables. The final variables are precipitation, soil temperature, pH, ammonia, and nitrite and nitrate.

	P	*ST*	*SM@10*	*SM@20*	*SM@30*	*pH*	*Amm*	*N&N*
P		-0.059	0.108	0.110	0.094	-0.091	0.012	-0.024
ST	0.000		-0.843	-0.755	-0.632	-0.340	0.024	0.348
SM@10	0.000	0.000		0.872	0.799	0.333	-0.028	-0.295
SM@20	0.000	0.000	0.000		0.864	0.234	-0.012	-0.116
SM@30	0.000	0.000	0.000	0.000		0.213	-0.009	-0.063
pH	0.000	0.000	0.000	0.000	0.000		-0.132	-0.151
Amm	0.000	0.000	0.000	0.001	0.056	0.000		0.294
N&N	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

Table-5.3.1: Pearson correlation coefficients (upper right) and p-values (lower left) between the climate and environmental variables of the North Wyke Farm Platform.

	PCA1	**PCA2**	**PCA3**	**PCA4**
Eigenvalues	2.476	1.557	1.115	0.98
Variance Explained (%)	30.947	19.467	13.935	12.244
Cumulative Variance Explained (%)	30.947	50.414	64.349	76.593
Precipitation (P)	0.064	-0.165	-0.642	0.570
Soil Temperature (ST)	0.312	-0.369	-0.362	-0.498
Soil Moisture at 10cm (SM@10)	-0.508	-0.054	0.128	0.276
Soil Moisture at 20cm (SM@20)	-0.467	-0.394	-0.120	-0.112
Soil Moisture at 30cm (SM@30)	-0.461	-0.434	-0.124	-0.183
pH	-0.321	0.252	0.192	-0.326
Ammonia (Amm)	0.144	-0.440	0.525	0.422
Nitrite and Nitrate (N&N)	0.293	-0.483	0.313	-0.144

Table-5.3.2: Results of principal components analysis with the eigenvectors for axes 1, 2, 3 and 4 for the between the climate and environmental variables of the North Wyke Farm Platform.

5.3.1b. Measures of Forage Quantity and Quality

The first two components were found to explain 74.62% of the total variance for the measures of forage quantity and quality. The absolute loadings for every measure were greater than 0.2 on either component one or two. Total N of dry matter was found to be weakly related to Total C of Dry Matter (0.159) and Average Sward Height (-0.207). These results suggest independency of each measure and therefore all three were retained for further analyses.

	Total N of Dry Matter	*Total C of Dry Matter*	*Average Sward Height*
Total N of Dry Matter		0.159	-0.207
Total C of Dry Matter	0.000		-0.049
Average Sward Height	0.000	0.256	

Table-5.3.3: Pearson correlation coefficients (upper right) and p-values (lower left) between the herbage parameters of the North Wyke Farm Platform.

	PCA1	**PCA2**
Eigenvalues	1.286	0.953
Variance Explained (%)	42.86	31.76
Cumulative Variance Explained (%)	42.86	74.62
Total N of Dry Matter	-0.674	-0.051
Total C of Dry Matter	-0.473	0.979
Average Sward Height	0.568	0.602

Table-5.3.4: Results of principal components analysis with the eigenvectors for axes 1 and 2 between the herbage parameters of the North Wyke Farm Platform.

5.3.1c. Measures of Cattle Production

71.187% of total variation was explained by the first two components and had eigenvalues above one (PCA1: 2.058; PCA2: 1.507) (Figure-5.3.5). Every measure of cattle production was found to be higher than 0.2 on the components. Final live weight was found to be very strongly and significantly correlated with cold carcass weight (r = 0.955) (Figure-5.3.6). Final live weight was retained. The principal components and Pearson's correlation analysis reduced the measures of cattle production to rate of weight gain, abattoir premium/penalties, final live weight and dead weight value.

	PCA1	**PCA2**
Eigenvalues	2.058	1.507
Variance Explained (%)	41.057	30.14
Cumulative Variance Explained (%)	41.057	71.187
Rate of Weight Gain	0.044	-0.277
Abattoir Premium/Penalties	0.333	-0.591
Final Live Weight	-0.666	-0.214
Cold Carcass Weight	-0.645	-0.276
Dead Weight Value	0.166	-0.673

Table-5.3.5: Results of principal components analysis with the eigenvectors for axes 1 and 2 for the measures of cattle production of the North Wyke Farm Platform.

	Rate of Weight Gain	*Abattoir Premium/Penalties*	*Final Live Weight*	*Cold Carcass Weight*	*Dead Weight Value*
Rate of Weight Gain		0.128	0.004	0.013	0.089
Abattoir Premium/Penalties	0.019		-0.249	-0.142	0.530
Final Live Weight	0.936	0.000		0.955	-0.014
Cold Carcass Weight	0.812	0.010	0.000		0.023
Dead Weight Value	0.103	0.000	0.795	0.670	

Table-5.3.6: Pearson correlation coefficients (upper right) and p-values (lower left) between measures of cattle production of the North Wyke Farm Platform.

5.3.1d. Measures of Lamb Production

The eigenvalues indicated that the first three components explained a total of 77.219% (Figure-5.3.2). The absolute loadings demonstrated that all five measures of lamb production should be retained for future analyses from the principal component analysis. Weak Pearson's correlations ($r < 0.5$) were found between associations of the measures, but independence of each was still assumed. These results suggest retaining all the measures of lamb production for further analyses.

	Rate of Weight Gain	*Abattoir Premium/Penalties*	*Final Live Weight*	*Cold Carcass Weight*	*Dead Weight Value*
Rate of Weight Gain		-0.001	-0.181	0.010	0.024
Abattoir Premium/Penalties	0.978		-0.002	0.381	0.376
Final Live Weight	0.004	0.942		0.234	-0.472
Cold Carcass Weight	0.876	0.000	0.000		0.261
Dead Weight Value	0.714	0.000	0.000	0.000	

Table-5.3.7: Pearson correlation coefficients (upper right) and p-values (lower left) between measures of lamb production of the North Wyke Farm Platform.

	PCA1	**PCA2**	**PCA3**
Eigenvalues	1.648	1.338	0.875
Variance Explained (%)	32.952	26.762	17.505
Cumulative Variance Explained (%)	32.952	59.714	77.219
Rate of Weight Gain	0.070	0.120	0.990
Abattoir Premium/Penalties	0.561	-0.266	-0.100
Final Live Weight	-0.328	-0.710	0.075
Cold Carcass Weight	0.408	-0.597	0.055
Dead Weight Value	0.637	0.239	-0.101

Table-5.3.8: Results of principal components analysis with the eigenvectors for axes 1, 2 and 3 for the measures of cattle production of the North Wyke Farm Platform.

5.3.1e. Summary of Principal Components of the North Wyke Farm Platform Data

The exploratory principal component analysis revealed that dimensionality reduction was applicable for the climate and environmental variables and measures of cattle production. The measures retained for further analyses were:

- *Climate and Environmental Variables:* precipitation, soil temperature, pH, ammonia, and nitrite and nitrate.
- *Measures of Forage Quantity and Quality:* average sward height, total C of dry matter and total N of dry matter.

- *Measures of Cattle Production:* rate of weight gain, abattoir premiums/penalties, cold carcass weight and dead weight value.
- *Measures of Lamb Production:* rate of weight gain, abattoir premium/penalties, final live weight, cold carcass weight and dead weight value.

5.3.2. Predicting the Provision of Forage Material from Temperate Grasslands

A subset regression was executed on the Park Grass Experiment and North Wyke Farm Platform data to indicate the mechanisms that support the provision of quantity (green biomass – average sward height from the NWFP data; brown biomass – dry matter yields from the PGE) and quality (total C and total N of dry matter from the NWFP data). The best models for each measure are shown in Table-5.3.9.

Measure of Forage Material	**Model Class**	**BIC**	**F[df]**	**R^2**	**Intercept**	**Selected Variable**	**Coefficient [SE]**
Quantity of Green Biomass	*Full*	609.303	$46.20_{[10,280]}$ ***	0.609	0±0.037	RNN MpH $MNND_{Seed}$ FDiv RpH MSoilTemp MNND MNN $Range_{CN}$ CWM_{Seed}	0.690±0.056*** 0.609±0.052*** -0.52±0.012*** -0.26±0.020** 0.241±0.043*** -0.20±0.051*** 0.199±0.071** -0.19±0.047*** -0.18±0.065** -0.11±0.042**
Quantity of Brown Biomass	*Enviro + Bio + Single*	1342.065	$84.2_{[12,647]}$ ***	0.602	0±0.025	Tot Fert App Rate CWM_{LNC} Tot Miner Add P Addition CWM_{CN} Minerals CWM_{LDMC} CWM_{Thick} $Range_{Thick}$ Grass Cover Fertilised $MNND_{LNC}$	1.10±0.092*** 0.644±0.098*** -0.53±0.092*** 0.519±0.056*** 0.450±0.098*** -0.44±0.062*** -0.37±0.073*** -0.33±0.080*** 0.157±0.036*** -0.14±0.034*** 0.108±0.036** -0.10±0.031**
Total Carbon of Brown Biomass	*Enviro + Single*	828.949	$9.486_{[5,285]}$ ***	0.055	0±0.057	MNN $MNND_{LNC}$	0.210±0.057** 0.157±0.057**
Total Nitrogen of Brown Biomass	*Enviro + Sin + Multi*	716.380	$26.93_{[5,285]}$ ***	0.417	0±0.045	RSoilTemp MAmmo $Range_{CN}$ MNN FRic RpH RNN $Range_{LDMC}$	-0.71±0.077*** -0.58±0.076*** -0.48±0.083** 0.475±0.062*** 0.393±0.088*** 0.280±0.072*** -0.19±0.056*** -0.17±0.052**

Table-5.3.9: Best model class for each measure of forage material together with the Bayesian Information Criteria and subset regression statistics. Abbreviations: BIC,

Bayesian Information Criterion; SE, Standard Error; MNN, Mean of Nitrite and Nitrate; RNN, Range of Soil Nitrite and Nitrate; Mammon, Mean of Soil Ammonia; MpH, Mean of Soil pH; RpH, Range of Soil pH; MSoilTemp, Mean Soil Temperature; RSoilTemp, Range Soil Temperature; Tot Fert App Rate, Total Fertiliser Application Rate; Tot Miner Add, Total Mineral Fertiliser Addition; P Addition; Triple Superphosphate; Grass Cover, Grass Coverage; MNND, Mean Nearest Neighbour Distance; CWM, Community-Weighted Mean; LDMC, Leaf Dry Matter Content; Seed, Seed Mass; Thick, Leaf Thickness; LNC, Leaf Nitrogen; C:N, Leaf C:N; FRic, Functional Richness; FDiv, Functional Divergence.

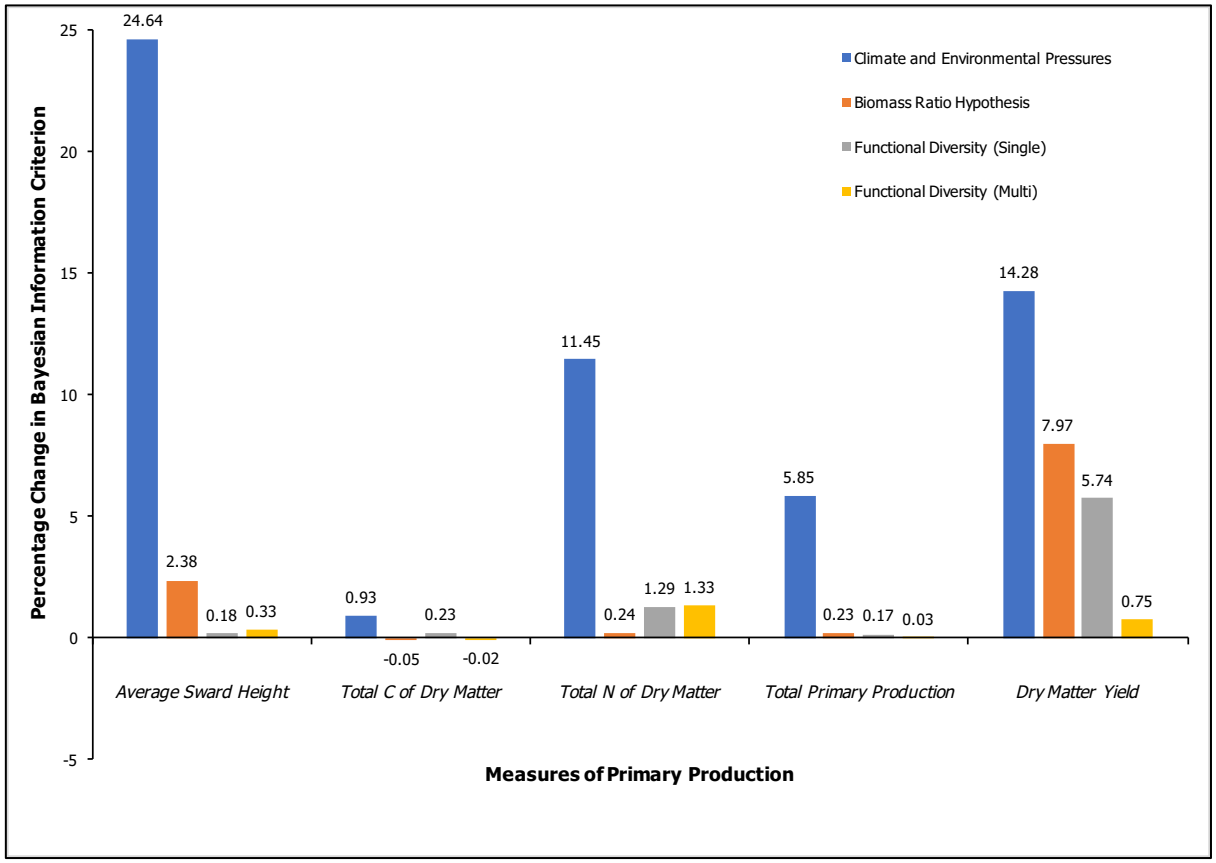

Figure-5.3.1. Percentage change in Bayesian Information Criterion across the measures of forage material for each single model class: climate and environmental pressures, biomass-ratio hypothesis, functional diversity (single), functional diversity (multi).

5.3.2a. Quantity of Green Biomass

Models developed to test the effects of different variable classes on the average sward height of the North Wyke Farm Platform (Table-5.3.10) were significant and the adjusted R^{2}'s ranged from 0.045 to 0.609. The best single-variable model class, as determined by Bayesian Information Criterion (BIC), was found to be the environmental effects model that also explained 57.4% of the total variation followed by the Biomass Ratio Hypothesis (15.4%), Functional Diversity (Multi) (6.2%) and Functional Diversity Single (4.5%).

For the two-variable model classes, when environmental variables were paired with Functional Diversity (Single) and Functional Diversity (Multi), the variables selected, the BIC and the adjusted R^2 remained unchanged from the single environmental effects model. However, the addition of the community-weighted mean of specific leaf area improved the BIC (from 610.916 to 609.661) and adjusted R^2 (0.574 to 0.583). The least effective two-factor model at explaining the variation in average sward height was found to be the pairing of Functional Diversity single and multi-trait indices (BIC= 821.064, adj R^2= 0.081). The inclusion of environmental effects to this two-factor model at the three-factor level showed a large increase in the amount of variation in average sward height that was explained – an increase of 50.2%.

At the three-factor level, the addition of Functional Diversity indices, single and multi-trait, showed no improvement in the environmental effects and Biomass Ratio Hypothesis combination. The poorest model at the three-factor level was found to be the three-way combination of the effect traits' models (Biomass Ratio Hypothesis and both Functional Diversity index classes).

The full model had the lowest BIC (609.303) and explained 60.9% of the variation in average sward height. The model indicates environmental variables > functional diversity (single) > functional diversity (multi) > biomass ratio hypothesis as their ranking according to coefficient values. This contrasts with the results across all models (Figure-5.3.1.) and Table-5.3.10), which suggests environmental variables

> biomass ratio hypothesis > functional diversity (single) > functional diversity (multi). Nonetheless, climate and environmental variables were the main determinant of green biomass production. The range of soil nitrite and nitrate has the highest coefficient value of all selected variables (0.69); across all models the range of soil nitrite and nitrate had the highest average coefficient (0.72) (Table-5.3.10). The mean and range of soil pH was also highlighted to be a key environmental factor. For effect traits, functional divergence at the single (seed mass and leaf C:N) and multi trait (FDiv and MNND) level was found to have a stronger impact on average sward height than the community-weighted mean of seed mass. However, inconsistencies were found for the effect of functional divergence measures on green biomass (positive: MNND, negative: FDiv).

Using the models outlined in Figure-5.1.3., the results suggest model four is the mechanism that best explains the production of green biomass as measured by average sward height. Overall, the greatest quantity of green biomass was produced by grasslands with cold soils, variation in acidity (pH levels below 7) and a range of nitrite and nitrate soil levels skewed at the lower level, which selected for plants species with smaller seeds and caused convergence in leaf C:N. These results highlight the importance of climate and environmental variables and seed mass as an effect trait controlling the production of green biomass.

Variable Class	**Selected Variables**	**Selection Rate**	**Average Coefficient [SE]**
Environmental Effects	RNN	100%	0.720±0.055
	MpH	100%	0.645±0.052
	RpH	100%	0.248±0.044
	MNN	100%	-0.20±0.048
	MSoilTemp	100%	-0.17±0.052
Biomass Ratio Hypothesis	CWM_{Seed}	62.5%	-0.22±0.032
	CWM_{SLA}	37.5%	0.041±0.016
Functional Diversity (Single)	$MNND_{Seed}$	12.5%	-0.07±0.012
	$Range_{CN}$	37.5%	-0.06±0.022
	FRO_{Seed}	12.5%	-0.04±0.009
	FRO_{Thick}	12.5%	0.022±0.009
Functional Diversity (Multi)	FRic	25%	-0.04±0.014
	FDiv	12.5%	-0.03±0.009
	βRao'sQ	12.5%	-0.03±0.007
	MNND	12.5%	0.025±0.009

Table-5.3.10: Selected variables, rates and average coefficients and standard error

across the fifteen models for average sward height for the North Wyke Farm Platform. Abbreviations: MNN, Mean of Nitrite and Nitrate; RNN, Range of Nitrite and Nitrate; MpH, Mean of Soil pH; RpH, Range of Soil pH; MSoilTemp, Mean of Soil Temperature; CWM, Community-Weighted Mean; FRO, Functional Regularity; MNND, Mean Nearest Neighbour Distance; FRic, Functional Richness; FDiv, Functional Divergence; SLA, Specific Leaf Area; Thick, Leaf Thickness; Seed, Seed Mass; C:N, Leaf C:N.

5.3.2b. Quantity of Brown Biomass

All models testing the yields of dry matter from the Park Grass Experiment were found to be statistically significant and explained between 5% and 60.2% of variation. At the single-variable model class, climate and environmental variables were superior to the hypotheses of effect traits. Multi-trait functional diversity indices, notably βRao'sQ and FDiv, explained the least amount of variation in yields of dry matter (5%). Unlike green biomass, single trait indices for the Functional Diversity and Biomass Ratio hypotheses were more effective in explaining yields of brown biomass. Community-weighted means and single evenness and divergence measures explained 42.4% and 41.2% respectively.

At the two-variable model class, the combination of any model class decreased BIC and increased adjusted R^2. Climate and environmental variables with community-weighted means explained 58.4% of total variation in dry matter yield; total fertiliser application rate and the application of triple superphosphate were found to have the highest coefficients together with community-weighted means of leaf nitrogen content and leaf C:N, which all had positive effects on brown biomass production. The least effective two-variable model was found to be the community-weighted means of plant height, leaf nitrogen content and specific leaf area with FDiv.

The inclusion of Functional Diversity single trait indices ($Range_{Thick}$ and $MNND_{LNC}$) was found to improve (reduction in BIC and increased adjusted R^2) model efficiency of the combination of climate and environmental variables and community-weighted

means. This model class had the lowest BIC (1342.065) and explained the most variation (60.2%) of all model classes. Total fertiliser application rate, community-weighted mean of leaf nitrogen content and application of triple superphosphate were found to have the strongest positive effects on the yield of dry matter from the Park Grass Experiment. This is also shown in Table-5.3.11. Despite triple superphosphate having a positive effect, the general application of mineral fertilisers (Total Mineral Addition and Minerals) was shown to have a negative influence. The best model indicates environmental variables > biomass ratio hypothesis > functional diversity (single) as their ranking according to coefficient values. This is consistent for the results across all models (Figure-5.3.1. and Table-5.3.11).

Using the models outlined in Figure-5.1.3., the results suggest Model Four, without multi-trait indices, is the mechanism that best explains the production of brown biomass as measured by dry matter yield. Overall, the greatest quantity of brown biomass was produced by fertilised grasslands, predominantly with triple superphosphate, but not with mineral fertilisers. These environmental conditions increased the coverage of non-grass species with thinner leaves, convergence skewed toward greater leaf nitrogen content levels and leaf C:N, and divergence skewed toward lower values of leaf dry matter content. These results highlight the importance of climate and environmental variables and leaf chemistry effect traits in producing brown biomass.

Variable Class	Selected Variables	Selection Rate	Average Coefficient [SE]
Environmental Effects	Tot Fert App Rate	87.5%	0.826±0.069
	P Addition	87.5%	0.456±0.048
	Minerals	87.5%	-0.35±0.055
	Tot Miner App Rate	62.5%	-0.29±0.058
	Grass Coverage	87.5%	-0.11±0.029
	Fertilised	62.5%	0.071±0.023
	Lime Addition	12.5%	-0.01±0.004
	Na Addition	12.5%	-0.01±0.004
	pH	12.5%	0.010±0.004
Biomass Ratio Hypothesis	CWM_{LNC}	75%	0.542±0.082
	CWM_{CN}	75%	0.307±0.071
	CWM_{PH}	75%	0.297±0.027
	CWM_{LDMC}	62.5%	-0.24±0.047
	CWM_{Thick}	50%	-0.21±0.049
	CWM_{SLA}	25%	-0.06±0.013
Functional Diversity (Single)	$Range_{CN}$	37.5%	-0.17±0.017
	$MNND_{Seed}$	37.5%	0.070±0.013
	$MNND_{Thick}$	37.5%	0.080±0.016
	$Range_{SLA}$	25%	0.079±0.014
	$Range_{PH}$	37.5%	0.075±0.021
	$MNND_{LNC}$	62.5%	-0.07±0.021
	$Range_{Thick}$	37.5%	0.068±0.013
	$MNND_{LDMC}$	12.5%	0.051±0.013
	$MNND_{CN}$	25%	-0.04±0.001
	$Range_{LNC}$	12.5%	-0.03±0.011
	$MNND_{SLA}$	25%	-0.03±0.010
	$MNND_{CN}$	12.5%	0.021±0.004
	$MNND_{Seed}$	12.5%	0.017±0.006
	$Range_{PH}$	12.5%	-0.01±0.004
	FRO_{CN}	12.5%	-0.01±0.005
Functional Diversity (Multi)	MNND	12.5%	-0.05±0.011
	FRic	25%	0.05±0.011
	βRao'sQ	25%	-0.05±0.011
	FDiv	37.5%	-0.05±0.011

Table-5.3.11: Selected variables, rates and average coefficients and standard error across the fifteen models for dry matter yield from the Park Grass Experiment. Abbreviations: Tot Fert App Rate, Total Fertiliser Application Rate; P Addition, Triple Superphosphate; Tot Miner App Rate, Total Mineral Application Rate; Na Addition, Sodium Sulphate; CWM, Community-Weighted Mean; FRO, Functional Regularity; MNND, Mean Nearest Neighbour Distance; PH, Plant Height; LDMC, Leaf Dry Matter Content; SLA, Specific Leaf Area; Thick, Leaf Thickness; Seed, Seed Mass; LNC, Leaf Nitrogen Content; C:N, Leaf C:N; FRic, Functional Richness; FDiv, Functional Divergence.

5.3.2c. Total Carbon of Brown Biomass

Variable Class	Selected Variable	Selection Rate	Average Coefficient [SE]
Environmental Effects	MNN	100%	0.205±0.058
Biomass Ratio Hypothesis	CWM_{Seed}	37.5%	-0.05±0.022
Functional Diversity (Single)	$MNND_{LNC}$	100%	0.138±0.058
Functional Diversity (Multi)	FRic	25%	0.023±0.015

Table-5.3.12: Selected variables, rates and average coefficients and standard error across the fifteen models for total carbon of brown biomass for the North Wyke Farm Platform. Abbreviations: MNN, Mean of Nitrite and Nitrate; CWM, Community-Weighted Mean; MNND, Mean Nearest Neighbour Distance; FRic, Functional Richness; Seed, Seed Mass; LNC, Leaf Nitrogen Content.

Of the fifteen models tested, nine models were found to be significant in explaining the total carbon of dry matter. These models, however, were weak and explained only 1.5-5.5% of the total variation. At the single-variable model class, soil mean nitrite and nitrate and divergence in leaf nitrogen content explained 3.4 and 1.5% respectively and both had positive impacts across all models (Table-5.3.12). Collectively, they were the best models in explaining total carbon dry matter with respect to the BIC (828.949). This result suggests Model Three, with multi-trait indices, is the mechanism that best explains the total carbon of brown biomass as measured by total carbon of dry matter. Overall, total carbon of dry matter increased with grasslands with higher soil nitrite and nitrate levels, which caused divergence in leaf nitrogen content. This result highlights the importance of soil fertility status and the functional divergence of leaf chemistry effect traits in determining the amount of carbon in dry matter yields. However, given the weak relationship (adjusted R^2= 0.055) found, this should be interpreted with caution.

5.3.2d. Total Nitrogen of Brown Biomass

Variable Class	Selected Variable	Selection Rate	Average Coefficient [SE]
Environmental Effects	RSoilTemp MAmmo MNN RpH RNN	100% 100% 100% 100% 50%	-0.72±0.078 -0.62±0.074 0.527±0.063 0.316±0.072 -0.08±0.029
Biomass Ratio Hypothesis	CWM_{Seed}	37.5%	-0.07±0.020
Functional Diversity (Single)	$MNND_{Thick}$ $MNND_{Seed}$ $Range_{CN}$ FRO_{Thick} $Range_{Thick}$ $Range_{LDMC}$	25% 25% 25% 50% 25% 25%	0.281±0.066 -0.21±0.066 -0.18±0.033 -0.16±0.045 -0.14±0.035 -0.04±0.013
Functional Diversity (Multi)	FRic βRao'sQ FEve	50% 75% 25%	0.186±0.047 0.175±0.042 0.105±0.032

Table-5.3.13: Selected variables, rates and average coefficients and standard error across the fifteen models for total nitrogen of brown biomass for the North Wyke Farm Platform. Abbreviations: MNN, Mean of Nitrite and Nitrate; RNN, Range of Nitrite and Nitrate; MAmmo, Mean of Ammonia; RpH, Range of Soil pH; RSoilTemp, Range of Soil Temperature; CWM, Community-Weighted Mean; FROm Functional Regularity; MNND, Mean Nearest Neighbour Distance; FRic, Functional Richness; FEve, Functional Evenness; LDMC, Leaf Dry Matter Content; Thick, Leaf Thickness; Seed, Seed Mass; CN, Leaf C:N.

Ten of the fifteen tested models were found to be significant and explained between 3.25% and 41.7%. The two insignificant models were functional diversity (multi) alone and in combination with community-weighted means. At the single-variable level, climate and environmental variables explained the most variation in total nitrogen of dry matter (32.5%) and recorded the lowest BIC (830.775). Effect traits, both community-weighted means and single/multi-trait functional diversity indices, explained less than 7.5% of the total variation in total nitrogen of dry matter.

At the two-variable model class, the combination of climate and environmental variables with single-trait functional diversity indices explained 37.5% of the total variation; range of soil temperature (-0.69±0.079) and the mean concentrations of ammonia (-0.59±0.079) and nitrite and nitrate (0.515±0.064) were found to have the highest coefficients together. The least effective two-variable model was found to be the community-weighted mean of seed mass with the evenness and range of leaf thickness.

The addition of functional richness was found to improve (reduction in BIC and R^2) model efficiency for the combination of climate and environmental variables and single-trait functional diversity indices. This model class had the lowest BIC (716.38) of all model classes and explained 41.7% of total nitrogen of dry matter from the North Wyke Farm Platform. Range of soil temperature, mean of ammonia, range of leaf C:N were found to have the strongest negative effects on the total nitrogen of dry matter. The strongest positive influence was found to be mean nitrate and nitrite. The best model indicated environmental variables > single trait functional diversity > multi trait functional diversity. This is consistent with the results across all models (Table-5.3.13.).

Using the models outlined in Figure-5.1.3., the results suggest Model Three is the mechanism that best explains total nitrogen of dry matter. Overall, dry matter with the greatest nitrogen content was produced by grasslands with high nitrate and nitrite and low variation in their levels, decreased soil ammonia that converged leaf dry matter content and leaf C:N. These results illustrate the role of climate and environmental variables and the functional diversity indices in the production of nitrogen rich dry matter.

5.3.2e. Summary of the Provision of Forage Material from Temperate Grasslands

This chapter found evidence for Response-Effect Framework in the provisioning of forage material; the significance of environmental effects and plant traits in the delivery of ecosystem processes and services. Environmental variables were found to have the greatest effect on all measures of forage material quantity and quality.

From the best models (Table-5.3.9.) and the change in the Bayesian Information Criterion (Figure-5.3.1.), measures of soil fertility status were found to be the most influential in both the North Wyke Farm Platform and the Park Grass Experiment. Soil nitrite and nitrate indicated a trade-off between forage quantity and quality. Increased nitrite and nitrate with little variation was associated with less forage material rich in protein. The importance of soil fertility was also highlighted in the Park Grass Experiment. In contrast to the North Wyke Farm Platform, fertilisation, specifically with triple superphosphate, increased brown forage material. These discrepancies may be attributed to the brown/green status of the forage material.

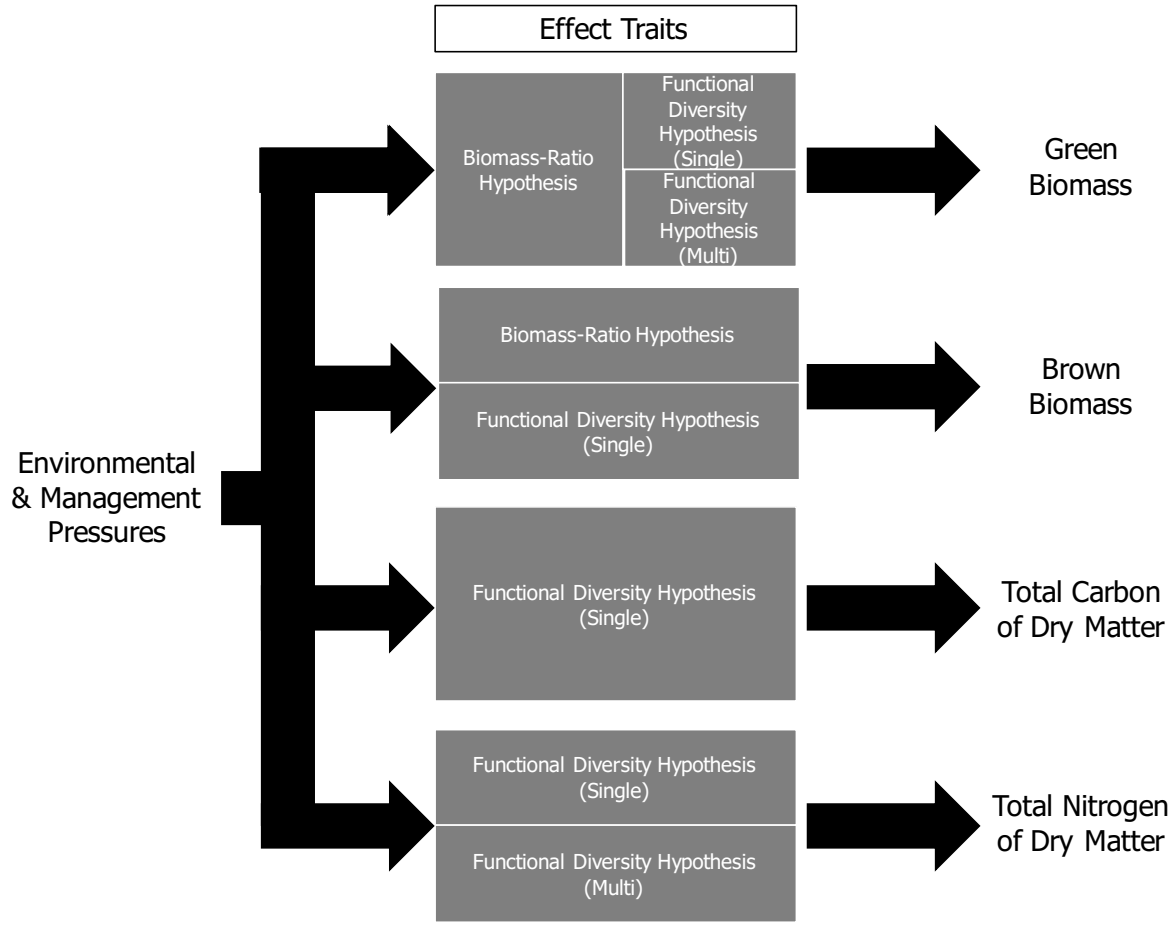

Figure-5.3.2. Summary of Response-Effect Framework in the context of the provision of forage material (quantity and quality). Environmental and management pressures were included in explaining each measure.

Secondary to environmental variables are the hypotheses of effect traits. The results of these hypotheses indicated that the Biomass-Ratio Hypothesis is of particular importance in explaining the delivery of forage quantity, whereas the Functional Diversity Hypothesis underpins forage quality (Figure-5.3.2.). This was also evident in the analyses of average coefficients across all fifteen models for average sward height, dry matter yield, total carbon of dry matter, and total nitrogen of dry matter. Dominant species with smaller seeds, faster relative growth rates and protein-rich leaves were found to produce greater amounts of green and brown biomass. Higher quality biomass was also produced from swards converged with respect to leaf C:N and leaf dry matter content. Although there is no indication of the direction of this convergence, one postulates it is towards lower values.

5.3.3. Assessing the Mechanisms of Livestock Production

The Lasso regression showed that no variables were selected for all measures of lamb production and rate of livestock weight gain, livestock abattoir premiums/penalties and livestock dead weight value (Table-5.3.14). The results demonstrate the importance of effect traits in predicting livestock and cattle production, and unlike parameters of forage quantity and quality, less emphasis is placed on climate and environmental factors. Additionally, the failure to select for forage quantity and quality factors in the Lasso regression shows effect traits are adequate for explaining variations in livestock and cattle production.

5.3.3a. Total Livestock Production

Explained variation measures of livestock production ranged from 42.6% to 94.5%. The different facets of livestock production were found to be best explained by the indices of the Functional Diversity hypothesis. Livestock final live weight was found to be positively associated with the range of plant height and was the most poorly explained parameter of livestock production (R^2= 0.426). The range of leaf dry matter content and leaf thickness had positive influences on the livestock cold carcass weight and this was better explained than final live weight (R^2= 0.833). Livestock units were explained by a combination of single and multi-trait indices; βRao'sQ was found to have a negative impact on livestock units whereas the

functional regularity of leaf thickness and functional evenness had positive associations.

These results highlight the importance of between-site functional divergence and within-site functional evenness, especially of leaf thickness, in dictating the number of livestock the North Wyke Farm Platform can support. In essence, stocking numbers are highest when the catchments have similar trait profiles and the within-catchment distribution of traits, particularly leaf thickness, are equal. Final live weight and cold carcass weight of livestock were positively driven by within-catchment functional divergence of plant height, leaf dry matter content and leaf thickness suggesting a functional variability in these traits produce heavier livestock.

5.3.3b. Cattle Production

The R^2s of measures of cattle production ranged from 0.242 to 0.999, and were best predicted by community-weighted means, multi-trait functional diversity indices and their combination. Between-catchment functional divergence was found to explain 24.2% of variation in rate of cattle weight gain, suggesting that rotational grazing with functionally dissimilar fields will increase the rate of cattle weight gain. 88.2% of the variation in cattle abattoir premiums/penalties were best explained by community-weighted means of seed mass (negative effect) and specific leaf area (positive effect); seed mass had a stronger effect. This suggests that beef cattle farmers should favour forage species with smaller seeds and higher relative growth rates (high specific leaf area) as this is likely to result in greater amounts of premiums, and thus economic gains. Cattle dead weight value was found to be negatively associated with FRic and FEve but positively associated with the community-weighted mean of leaf dry matter content, thus greater monetary value was produced from cattle grazed on grasslands with a smaller and less even niche space, which were dominated by plant species with high tissue density. The latter result contradicts the finding from abattoir premiums and penalties.

Cattle cold carcass weight and cattle stocking units were both positively associated with functional richness. The former was also negatively associated with

community-weighted mean of plant height and positively with functional divergence. These results suggest that greater niche space occupancy in grassland communities is conducive to greater stocking numbers of heavier cattle. Additionally, a diverse niche space dominated by smaller plants will support the production of heavier carcasses.

Measure of Livestock Production	**Model Class**	**R^2**	**Intercept**	**Selected Variable**	**Coefficients**
Livestock Final Live Weight	Sin	0.426	-0.037	$Range_{PH}$	0.222
Livestock Cold Carcass Weight	Sin	0.833	0.011	$Range_{LDMC}$ $Range_{Thick}$	0.951 0.148
Livestock Units	Sin_Multi	0.945	0.244	βRao'sQ FRO_{Thick} FEve	-0.203 0.164 0.055
Cattle Weight Gain	Multi	0.242	0.161	βRao'sQ	0.242
Cattle Abattoir Premiums/Penalties	Bio	0.882	-26.440	CWM_{Seed} CWM_{SLA}	-19.220 3.552
Cattle Cold Carcass Weight	Bio_Multi	0.996	310.133	CWM_{PH} FRic FDiv	-50.506 5.832 1.300
Cattle Dead Weight Value	Bio_Multi	0.999	370.294	FRic CWM_{LDMC} FEve	-3.832 3.787 -0.213
Cattle Stock Units	Multi	0.999	0.340	FRic	0.354

Table-5.3.14: Selected variables, model class and coefficients for the measures of total livestock and cattle production for the North Wyke Farm Platform. Abbreviations: CWM, Community-Weighted Mean; FRO, Functional Regularity; FRic, Functional Richness; FEve, Functional Evenness; FDiv, Functional Divergence; PH, Plant Height; LDMC, Leaf Dry Matter Content; SLA, Specific Leaf Area; Seed, Seed Mass; Thick, Leaf Thickness.

5.3.3c. Summary of the Provision of Livestock Production from Temperate Grasslands

Climate and environmental variables were not selected in the Lasso regression and the importance of effect traits was demonstrated for measures of livestock and

cattle production. Community-weighted means were found to only feature in the quantitative (cold carcass weight and stock units) and qualitative (abattoir premiums/penalties and dead weight value) measures of cattle production, but the significance of the functional diversity hypothesis especially multi-trait indices was demonstrated. The associations of functional richness elicited a key trade-off between cattle quantity and quality. Increased occupancy, and breadth, of the niche space produces more meat of lower quality and reduced economic value, thus suggesting functional redundancy is vital in providing adequate amounts of forage material to support beef production; but high-quality beef is underpinned by a small section of the total niche space. This is further echoed by beef quality being associated with an uneven niche space, whereby dominant species have small seeds, high tissue density, fast relative growth rates and are short in stature as implicated from cattle cold carcass weight.

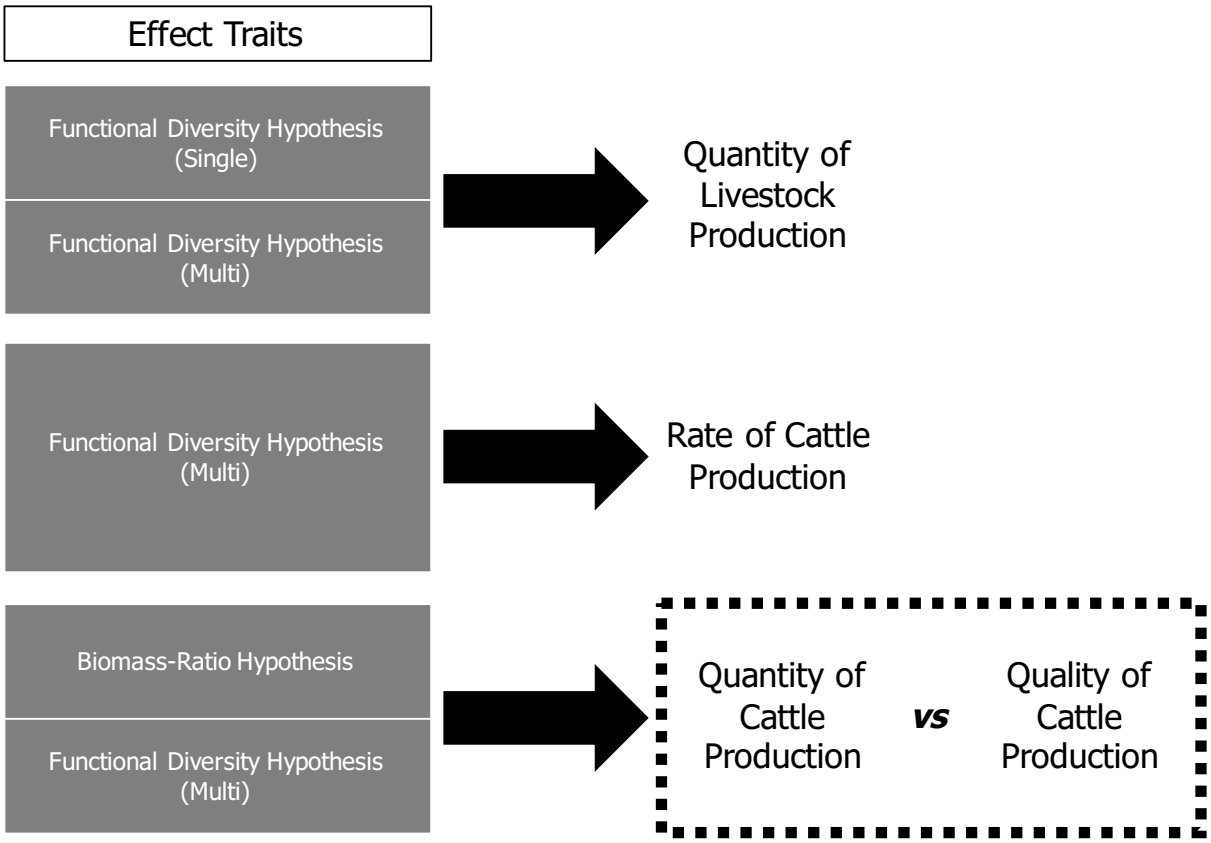

Figure-5.3.3. Summary of effect trait hypotheses on the provision of livestock and cattle production. The results indicated a trade-off in the quantity and quality of cattle production as highlighted.

5.4. Discussion

The impact of a temperate grassland's functional structure and composition on the provisioning of animal production has been severely understudied. Scholars have emphasised the effects of environmental and management practices on grazing pastures and the consequent effects at the consumer level (quantity and quality of meat and cheese products). The internal structure and mechanisms of this 'black box' that calibrates the environmental and management pressures with effect traits has been solely investigated by Pakeman (2014). This piece of research demonstrated a weak but positive influence of community-level leaf dry matter content and rainfall on secondary production (livestock units per hectare per year). The significance of community-weighted means, the Biomass Ratio Hypothesis, and leaf dry matter content has been noted for the delivery of forage material and subsequently livestock production via the inferred concepts of palatability and digestibility. The Biomass-Ratio Hypothesis is postulated to be a stronger determinant of ecosystem processes and services than measures of the Functional Diversity Hypothesis. However, researchers have indicated that neither hypothesis is satisfactory (Laliberté and Tylianakis, 2012; Funk *et al.*, 2016). This chapter aimed to clarify the scaling mechanisms of effect traits (Biomass Ratio vs Functional Diversity Hypotheses) in supporting the delivery ecosystem services from temperate grasslands – particularly biomass and food. Evidence for Lavorel and Garnier's Response-Effect Framework was revealed for the production of green and brown biomass, but the efficacy of environmental and management factors in explaining livestock production was challenged. Four hypotheses were proposed and each of these are discussed with reference to the key results (outlined in 5.3.2e. and 5.3.3c.) in the coming sections.

5.4.1. Quantity of Green and Brown Biomass

Literature seeking to understand the mechanisms that support the production of forage material has been highly fragmented. A range of scholars have individually highlighted the significance of different environmental variables, plant traits and functional diversity indices – quantifying both the Biomass-Ratio Hypothesis and the Functional Diversity Hypothesis (Tallowin and Jefferson, 1999; Clark *et al.*, 2012; Grigulis *et al.*, 2013; da Silveira Pontes *et al.*, 2015). It was hypothesised that

environmental and climate factors together with the community-weighted means would provide the best explain the greatest amount of variation in green and brown biomass production. The results of this chapter suggest a more holistic approach. incorporating environmental factors. and indices of both the Biomass-Ratio Hypothesis and the Functional Diversity Hypothesis explained circa 60% of total variation in green and brown biomass. Environmental and climate factors were shown to be the most influential factors on the quantity of brown and green biomass followed by effect traits. An illustrative model of the main results is presented in Figure-5.4.1, and the relative effects of environmental and climate variables (5.4.1b.) and effect traits (5.4.1c.) are discussed.

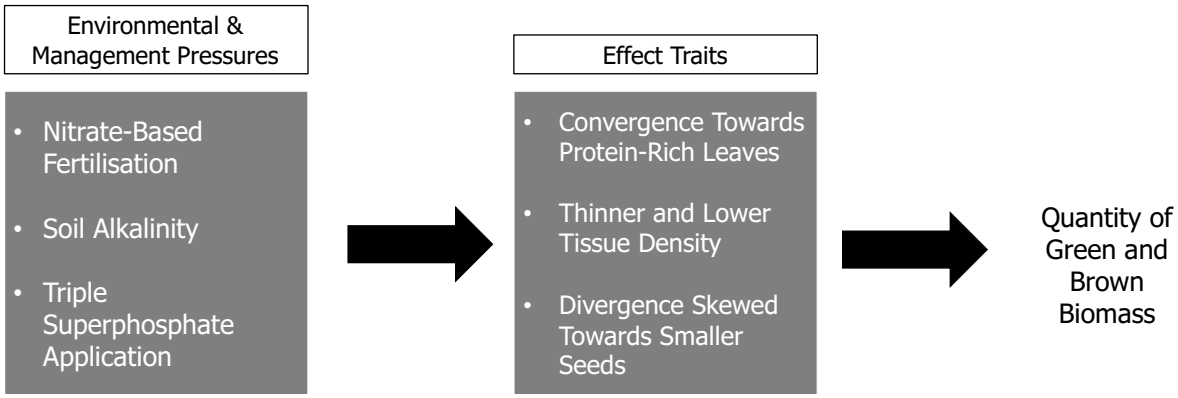

Figure-5.4.1. Summary of the main impacts of both climate and environmental factors and effect trait on the delivery of green and brown biomass from the Park Grass Experiment and the North Wyke Farm Platform.

5.4.1a. Relative Effects of Environmental and Climate Variables

Intensification of temperate grasslands occurred in the 19th and 20th centuries to increase total annual production (Pavlu *et al.*, 2007). Agricultural improvement using inorganic fertilisers (nitrogen, phosphorus and potassium) was found to increase the quantity and quality of grazing pasture via green biomass (Hopkins and Wilkins, 2006). Moreover, the addition of inorganic fertiliser increased dry matter yields (brown biomass) by 10 to 12 tonnes per hectare per year (Tallowin and Jefferson, 1999). The results of the North Wyke Farm Platform and the Park Grass Experiment support the use of inorganic fertilisers to boost the production of green and brown biomass. Across all sites, fertiliser application was a strong, and positive,

determinant of green and brown biomass. Specifically, nitrate-based fertilisers and triple superphosphate increased biomass whilst the application of other mineral fertilisers decreased green and brown biomass production. Further, soil pH skewed towards alkalinity supported production. These results echo the findings of literature published from the Park Grass Experiment. The combination of nitrogen-based fertilisation in combination with triple superphosphate was found to initiate a botanical shift conducive to biomass production (Silvertown *et al.*, 1994; Crawley *et al.*, 2005). Additionally, increasing soil alkalinity was found to stabilise interannual variation in hay biomass through increased resilience to adverse weather conditions (Dodd, Silvertown, Mcconway, *et al.*, 1994). Community resilience has been quantified through indices of the Functional Diversity Hypothesis under the assumption that a broad and evenly saturated niche space guarantees sustainability of community structure and function (Mason *et al.*, 2005).

5.4.1b. Relative Impacts of Effect Traits

Plant-life form, such as Raunkiær' classification, was an early discrete trait that inspired investigations into individual effect traits (Rihawi *et al.*, 1987). The scaling of plant traits to biomass production has suggested the Biomass-Ratio Hypothesis as the leading mechanism and this was hypothesised in this chapter (da Silveira Pontes *et al.*, 2015). The results of this chapter indicate the importance of both hypotheses in explaining the production of biomass from temperate grasslands and supports Clark *et al* (2012) in advocating an integrated approach. Nonetheless, the results demonstrated differing explanatory powers of the hypotheses; the Biomass-Ratio Hypothesis for brown biomass and the Functional Diversity Hypothesis for green biomass. The coefficients of the best subset models revealed that the Biomass Ratio Hypothesis was about six times stronger in explaining brown biomass and the Functional Diversity Hypothesis five times stronger for green biomass. These findings, therefore, partially satisfy Hypothesis One of this chapter and challenge a number of publications; Garnier *et al* (2004), Duru *et al* (2010), Laliberté and Tyliankis (2012) and Roscher *et al* (2013). It is clear, however, that both hypotheses contribute to the production of biomass from temperate grasslands.

The Functional Diversity Hypothesis is premised on the concepts of functional redundancy and insurance. It assumes that high functional diversity guarantees community stability and the provision of ecosystem services (Yachi and Loreau, 1999). Examining the direction of the univariate and multivariate functional diversity indices in the best models generally contradicts the Functional Diversity Hypothesis. Divergence had a negative impact on green biomass production at the multivariate (FDiv) and univariate (seed mass and leaf C:N) levels, and divergence in leaf nitrogen content resulted in decreased brown biomass. This also counters the results of Kelemen *et al* (2015) who found a positive impact of plant height and seed mass divergence on green biomass. This chapter questions the applicability of the Functional Diversity Hypothesis in explaining biomass production and single ecosystem processes/services. The current hypothesis is likely to be more pertinent to explaining the multifunctionality of temperate grasslands. Multifunctionality, in this case, describes the balance between ecosystem processes and functions, such as primary production, biodiversity conservation, and nutrient retention (Zhang and Schwärzel, 2017). Despite this, this chapter advocates the use of univariate functional diversity indices to enrich the study and modelling of single ecosystem processes and services, because these indices, when combined with community-weighted means, unearth the ideal structure and composition of biomass producing temperate grasslands. In order to recreate or restore such system, a comprehensive illustration of the plant trait patterning needs to be defined.

Plant height has been at the forefront of primary production research and its significance at varying spatial scales has been reported (Laliberté and Tylianakis, 2012; da Silveira Pontes *et al.*, 2015). Plant height, however, did not feature here in the best models from the subset regression to explain the production of green or brown biomass. This challenges the focus on plant height as the principal determinant of biomass. Instead, communities with the greatest green and brown biomass resembled assemblages dominated by fast germinating (small seeds) with thin protein-rich leaves. Lighter seeds suggest that quick germination and establishment are key ecosystem properties that determine biomass production, although smaller seeded communities are traditionally viewed as unsustainable because maternal investment is directed towards seed output rather than the number and survivability of seedlings (Grime *et al.*, 1997; Weiher *et al.*, 1999). This

challenges the long-term viability of the production system and is further elucidated in the examination of the leaf traits. The foliar traits exhibited patterning typical of the exploitative strategy of the Leaf Economics Spectrum (Wright *et al.*, 2004). Thin protein-rich leaves were found to be indicative of enhanced green and brown biomass, and these results are congruent with preceding research that found a positive impact of leaf nitrogen content and the negative effects of leaf dry matter content (Garnier *et al.*, 2004; Duru *et al.*, 2010).

5.4.2. Quality of Brown Biomass

Quality of forage and livestock feed has commonly been used as a proxy for estimating food production. Research has, thus, neglected the *in-situ* measurement of biomass quality parameters, such as total carbon and total nitrogen. Instead, the concepts of palatability and digestibility have defined a suite of plant traits conducive to the production of quality biomass to support livestock production. Many studies have failed to adequately isolate the role of environmental and management pressures and the effect trait hypothesis in governing the quality of brown biomass. It was hypothesised that the Biomass-Ratio Hypothesis would be the dominant explanatory factor and that environmental factors would play a secondary role. The findings of this chapter, however, suggest the environmental and management factors are the leading forces followed by the convergence/divergence patterning of effect traits (the Functional Diversity Hypothesis). The best models significantly explained between 5% and 40% of the total variation in the quality of brown biomass. The model of total nitrogen of dry matter was found to be the superior explained model and will form the basis in discussing the relative effects of environmental and management factors and the Functional Diversity Hypothesis as illustrated in Figure-5.4.2.

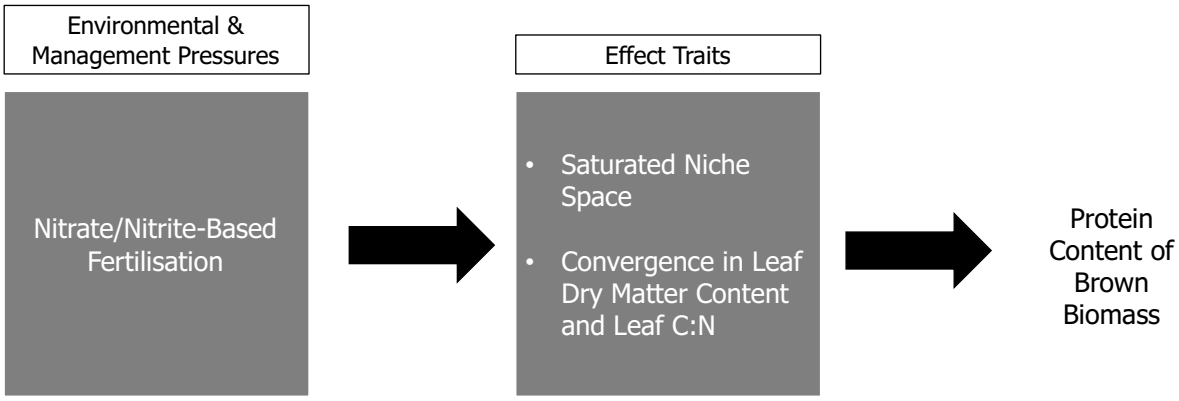

Figure-5.4.2. Summary of the main impacts of both climate and environmental factors and effect trait on total nitrogen (protein content) of brown biomass from the North Wyke Farm Platform.

5.4.2a. Relative Effects of Environmental and Climate Variables

The improvement of temperate grasslands has been shown to increase the palatability and digestibility of biomass. Palatable and digestible constituents have been found to be mediated by environmental and management pressures relating to nitrogen-fertilisation; community crude protein was found to be mediated by the soil nitrogen levels and responded positively to nitrogen fertilisation (Buxton, 1996; Tallowin and Jefferson, 1999). The results presented in this chapter align with the body of research, and further elucidate the role of nitrogen fertiliser origin (nitrate/nitrite or ammonia/ammonium). Specifically, nitrate or nitrite-based fertilisers have the potential to increase total nitrogen of dry matter, whereas ammonia or ammonium-based fertilisers have a negative impact.

5.4.2b. Relative Effects of the Functional Diversity Hypothesis

The quality of biomass has traditionally been deduced from community-level estimates of plant traits inferred by the concepts of palatability and digestibility (Ansquer *et al.*, 2009b; Mkhize *et al.*, 2014). This approach has resulted in the lack of *in situ* quality recordings, such as total nitrogen (crude protein) and total carbon of dry matter. Instead, research has accepted the use of community-weighted means of physiological traits, structural, non-structural and mineral components as surrogates for biomass quality. This chapter, however, demonstrated that the

Biomass-Ratio Hypothesis is a poor predictor of total nitrogen of brown biomass, and the Functional Diversity Hypothesis is the prominent mechanism. In line with this hypothesis, a saturated multivariate niche space (FRic) was found to support the production of protein-rich biomass. This postulates that niche complementarity is a positive driving factor in the provisioning of high quality biomass (Yachi and Loreau, 1999). A greater occupied niche space ensures a consistent quality of biomass irrespective of volume. Contrary to the Functional Diversity Hypothesis, temperate grassland communities converged with respect to leaf dry matter content and leaf C:N were found to produce high quality biomass, and this echoes the discussion of the Functional Diversity Hypothesis with regards to biomass quantity (5.4.1b.).

5.4.3. Managing Temperate Grasslands for Biomass Production

Collectively, the findings advocate for the intensive management of temperate grasslands to increase both the yield and quality of biomass production. Specifically, nitrate-based fertilisers would be the most efficient, particularly when combined with triple superphosphate and regular soil pH examinations to maintain alkalinity. This supports decades of research arising from the Park Grass Experiment (Silvertown *et al.*, 1994; Crawley *et al.*, 2005). Drawing on the Park Grass Experiment, nitrate-based fertilisation at rate between 96-144 kgN/ha/yr in combination with triple superphosphate would increase biomass yield and, provided soil pH remained alkaline, inter-annual variability in yields would be stable (Dodd, Silvertown, Mcconway, *et al.*, 1994; Tautenhahn *et al.*, 2008). The economic gains from this management strategy were estimated by Hodgson *et al* (2005) to be in the region of 200-600%; however a comprehensive analysis incorporating climate and environmental variables, plant traits, measures of biomass quantity, quality and value is lacking. This should be a focus of future studies in examining the provision of biomass from temperate grasslands.

5.4.4. Animal Production from Temperate Grasslands

Plant trait-based analyses to improve understanding of the delivery of livestock production have been severely lacking. Pakeman (2014) has been the sole

publication in functional ecology and this paper defined secondary productivity as the livestock units per hectare per year to achieve a set vegetation height. Pakeman investigated the effects of climate variables (mean temperature and rainfall), the Biomass-Ratio Hypothesis (community-weighted means of specific leaf area and leaf dry matter content) and the Functional Diversity Hypothesis (univariate and multivariate indices: FEve and Rao's Q). The paper reported that the combination of rainfall and the community-weighted mean of leaf dry matter content best explained livestock productivity, and thus informed hypotheses three and four of this chapter that climate and environmental variables and dominant species with palatable and digestible traits will play a significant role in explaining livestock production.

5.4.4a. Livestock and Cattle Units

The comparable results of this chapter (livestock and cattle units) disprove this hypothesis and challenge Pakeman's conclusions. The results from the North Wyke Farm Platform revealed that circa 95% of livestock productivity, defined as livestock and cattle units per hectare per year, is adequately explained by the univariate and multivariate indices of the Functional Diversity Hypothesis. These findings challenge Pakeman's conclusion that functional diversity is a poor predictor of livestock production and highlights the Functional Diversity Hypothesis as the leading mechanism. In essence, the findings revealed that livestock production is greatest from homogenous landscapes (low βRao's Q) consisting of individual grazing pastures that are functionally even with regards to their trait profiles and species abundances (high FEve), particularly leaf thickness. The positive impact of within-site functional evenness suggests that an equally utilised niche space leads to greater provision of livestock productivity, and supports the inferences made by Mouillot *et al* (2005) on the translation of functional evenness to ecosystem processes and services. Further evidence for the Functional Diversity Hypothesis was found in the positive effect of functional richness on cattle units, and aligns with previous scholars' assumptions of niche complementarity ensuring sustained delivery of ecosystem functions (Schleuter *et al.*, 2010).

Despite this, employing livestock units as a surrogate for quantity of livestock productivity can be criticised. Livestock units are traditionally a measure of grazing pressure and this exposes an underlying issue with implying causation (Soussana *et al.*, 2004). Instead, the results may reflect the effectiveness of the rotational grazing in the North Wyke Farm Platform. In essence, the results may suggest that the grazing intensity is consistent across the landscape, producing pastures that are both productive and sustainable. This is evident in the low between-site divergence and high within-site functional richness and evenness. Thus, it could be said that the managers of the North Wyke Farm Platform have adopted a proficient approach to rotational grazing. However, the implications of this on livestock production still remain unclear and therefore this chapter has investigated a range of more intricate measures of quantity, together with rate and quality of cattle production.

5.4.4b. Sophisticated Measures of Livestock and Cattle Production

5.4.4bi. Quantity and Rate of Production

This chapter employed final live and cold carcass weights of livestock as more detailed measures of livestock production. It was found that functionally diverged communities, particularly with regards to plant height, leaf dry matter content and leaf thickness, produced heavier livestock. This is logical given the composite nature (sheep and cattle) of these measures and is thought to reflect the interspecific differences in the grazing behaviours and physiology of cattle and sheep. Cattle and sheep have been categorised as 'grazers' and 'browsers', respectively, based on their morphophysiological characteristics. Gordon and Prins (2008) suggested that this characterisation is often unsubstantiated and suggested food intake and ingesta retention are critical differentiating factors. In light of this, the size of a grazer's mouthparts (lips, tongue and teeth) has been linked to the rate, quantity and quality of intake. Shipley *et al* (1999) further related bite size to simple measures of plant structure (diameter and mass) that could be interpreted from a plant trait perspective. In essence, bite size was postulated to represent the trade-off between quantity of ingestion with rate and quality. Larger bites are suggested to efficiently ingest greater amounts of calorific dry matter as there are fewer interruptions in chewing to crop new bites, however this is compensated by the lack of selectivity in grazing and the higher consumption of unpalatable and indigestible plant species

and/or organs (Gross *et al.*, 1993; Shipley *et al.*, 1999). Cattle and sheep are thought to sit at opposite ends of the bite size spectrum; sheep's smaller mouthparts prompts the adoption of 'browser' behaviours and the consequent ingestion of high quality forage (Rook *et al.*, 2004). This supports the segregation of cattle and sheep into differing grazing behaviours based on their morphophysiological characteristics that could be underpinned by the plant trait profile of the forage (Shipley *et al.*, 1999; Gordon and Prins, 2008). Research into understanding the plant trait optima for grazers and browsers is considerably lacking. However, what can be deduced from this chapter is that supplying a forage system that is diverged in respect to plant height, leaf dry matter content and leaf thickness will support the production of heavier cattle and sheep in a mixed herd system. This consequently offers direct support for the Functional Diversity Hypothesis and the traditional management of grazing pastures with promotion of mixed swards.

Spedding (1971) suggested that an optimal livestock diet cannot be provided by one single species and so the use of mixed forages is currently the norm (Rutter, 2010). Illius *et al* (1999) reported increased forage intake rate from mixed swards and it has been suggested that offering a diverse diet has the potential to improve livestock production (Rutter, 2010). Dietary diversity here has largely been interpreted from a taxon-focussed approach and has limited the breadth of species examined. Many studies have fixed on *Trilfolium spp* (clovers) and grass species; sheep and goats have been found to prefer clovers to grass species (Rutter, 2006). These mixed swards have been linked to increases in intake rates, thus production, in lambs via the avoidance of incidental restriction and augmentation (Raubenheimer, 1992; Catanese *et al.*, 2013). Raubenheimer (1992) defined these phenomena in support of instilling diversity in grazing forages/pastures. In essence, unbalanced forage limits the intake rate of livestock through the cessation of grazing once consumption of the limiting resource, such as protein, has been satisfied (incidental restriction), or the overconsumption to satisfy multiple nutritional demands (incidental augmentation) (Raubenheimer, 1992). In both cases, a decrease in fitness and productivity is expected; restriction via malnutrition and augmentation through the additional metabolic costs to digest excessive ingested forage (Soder *et al.*, 2007). Therefore, it could be assumed that the plant

communities at the North Wyke Farm Platform are functionally diverged enough to support cattle and sheep production. However, Gregorini *et al* (2017) noted a general lack of knowledge into the specific effects of dietary diversity on cattle production.

The present analysis found that heavier cattle were produced from functionally diverse communities. The positive effects of within-community functional diversity (FRic and FDiv) demonstrates that a diverged and saturated plant functional niche space produces heavier cattle. This provides further evidence for the Functional Diversity Hypothesis and echoes the results from the quantity of livestock production analyses and the benefits of dietary diversity on production. A particularly novel result is the increase in rate of cattle weight gain with between-community divergence (βRao'sQ) and, combining this with within-community functional diversity, it could be postulated that cattle production is optimised, in terms of rate and quantity, from a heterogeneous grazing landscape consisting of dissimilar functionally diverged communities (Figure-5.4.3). This assumption is based on the phenomenon known as sensory-specific transfer effects, that speculates consumers have a broad aversion to monocultures and foods that share similar sensory and nutritional characteristics. For example, it has been reported that ingestion rate decreases with sensory similarity (Rolls *et al.*, 1981). Applying this to cattle, the rate and amount of weight gain is optimised when individual plant communities are diverse enough to meet energy and protein requirements but are dissimilar enough from other pastures to maintain grazing vigour. Meuret and Provenza (2015) alluded to this phenomenon calling it 'grazing weariness' and this chapter supports their conclusions.

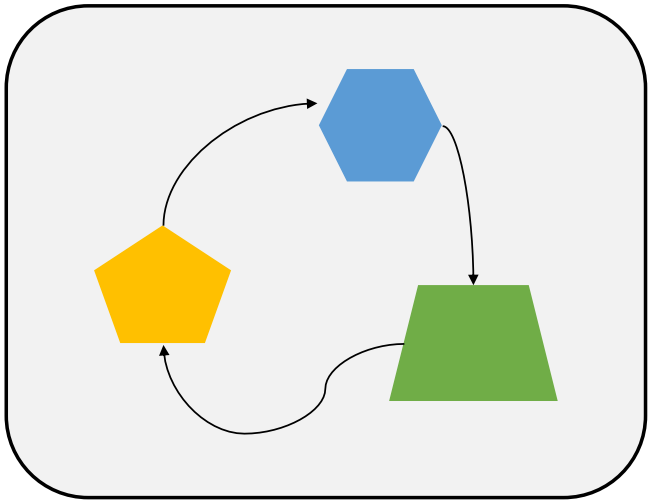

Figure-5.4.3. Abstract grazing landscape whereby each polygon represents an individual diverged community so that both between- and within-community functional diversity is high.

5.4.4bii. Quality of Cattle Production

The indirect link between botanical composition and the quality of cattle products has been realised following research on improved and semi-improved grasslands (Coulon *et al.*, 2004). It was postulated that plant species-specific chemical compounds, such as terpenes and carotenes, have the capacity to affect milk protein and fat in such a way as to adversely change the taste and odour of cheese. In light of this, hypothesis four stated that compounds associated with nutritional value, such as protein content, and climate and environmental factors governing their levels, would determine the quality of livestock products. The findings of this chapter counter this hypothesis as climate and environmental variables failed to be selected in the lasso regression. It is clear, however, that there appears to be a trade-off between producing heavier cattle and high-quality meat. Within-site functional richness and evenness was found to increase the financial penalties from abattoirs and decrease the dead weight value of cattle. This contradicts the current interpretation of the Functional Diversity Hypothesis, and the research suggesting that dietary diversity and phytochemical richness confer higher quality meat products (Gregorini *et al.*, 2017). This chapter suggests that a small number of plant traits with specific optima govern high-quality meat production, and this explains the negative impact at the multivariate scale. The identity of these traits is

yet to be defined within the literature, but the result for the community-weighted means may indicate a list of potential traits.

It was found that communities dominated by small seeded species with a high relative growth rate and leaf tissue density supported the delivery of high quality cattle meat. This trait profile, however, is largely analogous to current literature. Communities with high leaf tissue density would traditionally be deemed as difficult to digest (Buxton, 1996). The indigestible forage would induce bulk limitation by reducing ingestion, decreasing the efficiency of digestion and the adsorption of key nutrients (PENNING *et al.*, 1994). Inconsistencies between this study and published literature were also found in the results for the quantity of cattle production. In this study, communities dominated by short plants produced heavier cattle, which is implausible given the short bite depth of cattle (Illius and Gordon, 1987). This chapter, therefore, suggests the presented results for the community-weighted means should be interpreted with caution.

5.4.5. Managing Temperate Grasslands for Livestock Production

Taking into account livestock/cattle quantity and quality, it is clear that communities with moderate functional diversity (richness, evenness and divergence) best support a balanced system of livestock production. Gregorini *et al* (2017), however, envisaged a more isolated view of pastoral livestock production systems that creates a system accommodating for interspecific grazing and dietary preferences. A novel approach has been realised in France in the design of grazing foodscapes consisting of isolated monocultures that constitute a rotational grazing circuit (Meuret and Provenza, 2015).

Foodscapes are based on the engineering of homogenous swards to form rotational grazing circuits. Dividing mixed swards into homogenous communities was found to motivate grazing through the avoidance of weariness (Meuret and Provenza, 2015). The results of this chapter provide support for this management strategy; the rate of cattle weight gain was found to be positively related to landscape heterogeneity. Additionally, the quality and value of cattle meat was found to be highest from

functionally poor, and likely homogenous, communities. These findings, therefore, advocate the use of grazing circuits in livestock production systems to boost the rate and quality of livestock production. Increases in milk yield (11%) have also been reported from cattle rotated on homogenous grazing pastures (Edwards, Parsons and Bryant, 2008). The findings from this chapter, however, challenge Edwards, Parsons and Bryant's (2008) assumptions; cattle cold carcass weight increased with functional richness and divergence.

The timing and sequence of the rotations would need closer examination to optimise grazing vigour. Meuret and Provenza (2015) suggested livestock show temporal dietary preferences through diurnal patterning of forage intake. Livestock were found to ingest greater volumes of *Trifolium spp* (clover) in the morning, which was progressively substituted for grass species throughout the day (Penning *et al.*, 1997; Rutter, 2010). It is postulated that this patterning mirrors the nutritional and metabolic demands of the livestock, however, rigidly defining these needs is yet to be comprehensively achieved at the species-level. For functional ecology, there is potential to classify livestock into grazing guilds according to interspecific differences in metabolic demands. Spedding (1971) speculated that different protein requirements of cattle and ewes could enable the engineering of plant communities conducive to production. Cattle with a mean weight of 500kg were found to require a minimum of 3.1% of digestible crude protein in dry matter, whereas ewes (70kg) needed 4.3% to maintain their weight (Spedding, 1971). These results describe the interspecific metabolic demands and identify a potential herbivore trait profile to underpin the construction of guilds. However, this trait can be viewed as 'hard' (difficult to operationalise and measure) and could be simplified by physiological and morphological features, such as body mass or size of mouthparts (teeth, tongue, lips) (Shipley *et al.*, 1999). Furthermore, herbivores are often treated as mechanistic beings that lack the ability to adapt randomly, despite optimal foraging theories suggesting the opposite, for example orosensorial and post-ingestive feedback or experiences have the capacity to alter subsequent grazing behaviours (Mehiel, 1991). Furthermore, Villalba *et al* (2015) showed that grazing experiences *in utero* or early life may cause permanent behavioural changes. Young goats reared on rangeland dominated by blackbrush (*Coleogyne ramosissima*) were found to eat over 2.5 times more blackbrush than naïve goats

(Distel and Provenza, 1991). In this case, their rumen volume showed adaptation to cope with the higher tannin content and led to speculation that plant communities have the capacity to induce epigenetic, and heritable, adaptations in the short term, leading to locally specialised grazers – ecotypes (Villalba *et al.*, 2015). This is yet to be widely corroborated for domesticated herbivores but has been observed in other mammals, such as *Orcinus orca* and *Tursiops truncates* (Perrin *et al.*, 2011; Riesch and Deecke, 2011). For now, it is suggested that it would be worthwhile to investigate herbivore guilds and define a core list of animal traits, to include bite size, rate of intake, rumen capacity and digestion efficiency, and combine these with plant traits and measures of livestock production as illustrated in Figure-5.4.4.

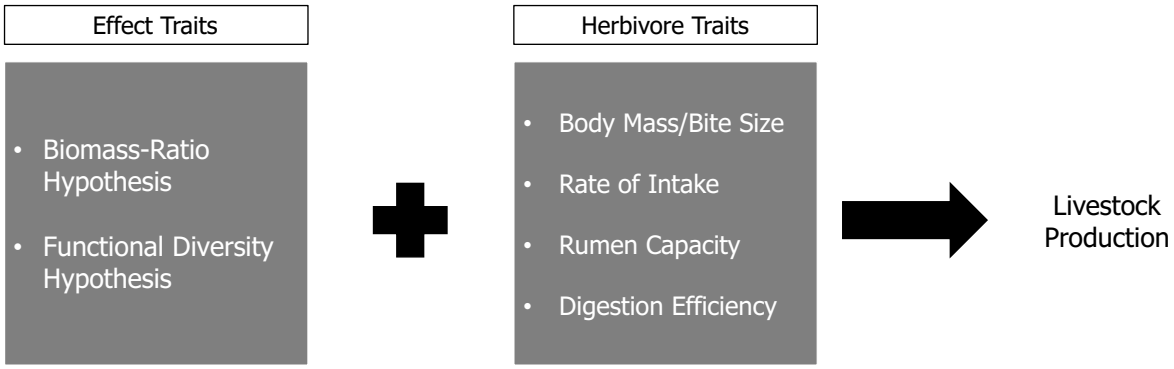

Figure-5.4.4. Suggested future analyses based on the amalgamation of plant effect and herbivore traits to predict livestock production.

5.4.5. Future Directions

5.4.5a. Expansion of Effect Traits and Ecosystem Services Research

Plant-trait research has focussed heavily on the delivery of a limited set of ecosystem services. Scholars have indicated inconsistencies in the literature with regard to the impact of effect traits on ecosystem services (Ricketts *et al.*, 2016). For example, supporting services are greatly underrepresented, Ricketts *et al* (2016) suggested that regulating services are likely to increase with functional diversity, provisioning services are dependent on abundance of harvested species and cultural services are underpinned by specific features in the landscape. The need for service-orientated grassland management remains a major challenge for trait-based approaches because of reductionism focussing solely on the floristic

community. Many ecosystem services are the result of the amalgamation of multi-trophic level interactions. The combination of plant functional composition (community-weighted means and functional diversity indices) and functional parameters of the soil microbiota were studied by Grigulis *et al* (2013) to understand the role of differing trophic levels in the provisioning of soil carbon sequestration and nitrogen retention from three grassland sites. They found the functionality of both trophic levels were equally important in supporting the delivery of these services. Pollination services are also an example of trophic interaction as the adequate delivery of pollination services is dependent on the interplay of floral and pollinator functional composition.

5.4.5b. Pollination Services

Understanding the provisioning of pollination services is vital, as 84% of global crop species require pollination by animals (Klein *et al.*, 2007). Managed and native bee pollination has been at the forefront of research and the importance of non-crop plants in temperate grasslands to support agricultural production has been realised (Hoehn *et al.*, 2008). Temperate grasslands provide nesting and overwintering sites as well as maintaining a consistent supply of nectar for *Bombus spp* during resource bottlenecks typical of colony foundation in early spring and colony reproduction in mid-late summer (Williams *et al.*, 2010; Rundlöf *et al.*, 2014). Pollen has been highlighted as a limiting resource for wild solitary and bumble bee populations (Larsson and Franzén, 2007). Identifying the complementary traits between plants and bees has been suggested as an important avenue for Functional Ecology and a holistic trait-study is suggested here as the next great breakthrough. The integration of trait-based analyses across different trophic levels has the capacity to benefit the management and restoration of temperate grasslands and advance the understanding of pollination syndromes – the co-evolved relationship between reproductive traits and pollen vectors (Dafni, Lehrer and Kevan, 1997; Reiss *et al.*, 2009). This section reviews the literature on plant functional traits and bee guilds and draws out a series of inter-dependent traits that could be used to predict and engineer the provisioning of pollination services from temperate grasslands. The plant-bee traits are separated into three classes based on bee foraging behaviour: attraction, visitation and revisitation (Figure-5.4.5.).

	Plant Trait	Bee Trait
Attraction	Plant Height Flower Shape, Symmetry and Pigmentation Floral Size and Density	Visual Cues
Visitation	Corolla Tube Length/Width	Bee Size Probiscus Length/Width
Revisitation	Pollen Grain Size Nectar Sugar Concentration	Metabolic Demands

Figure-5.4.5. Summary of three bee foraging behaviours with appropriate, and interdependent, plant and bee traits.

Morphological and chromatic visual cues are thought to attract bees to foraging sites initially (Gonzalez *et al.*, 1995). Plant height and flower size have been highlighted as attractive plant traits, based on their associations with rewarding units, such as pollen and nectar (Dafni, Lehrer and Kevan, 1997). It is surmised that larger floral displays on large species act as pollinator magnets, which facilitate community-level pollination, due to the positive relationship between size traits and nectar production (Cresswell and Osborne, 2004; Hegland and Totland, 2005; Gómez *et al.*, 2008). At finer scales, visual cues are key in the learnt preferences for floral shape, form, symmetry and pigmentation, which are suggested to be distinct for individual species of bee (Pernal and Currie, 2002; Lázaro, Hegland and Totland, 2008). For example, *Apis mellifera* (honeybee) were found to prefer purple flowers, whereas solitary bees most frequently visited yellow flowers (Lázaro, Hegland and Totland, 2008). Furthermore, solitary bees from the genus *Osmia* are thought to use floral cues to gauge pollen availability and adapt their foraging behaviour accordingly (Kraemer, 2001). Bumblebees (*Bombus spp.*) were found to commonly visit blue, pink or purple flowers and preferred patches where stalk/flower height was consistent (high functional evenness) because hovering expends less energy than taking a variable flightpath (Schmitt, 1983; Raine and Chittka, 2007). These visual cues are acquired throughout a bee's life cycle and increase foraging efficiency.

Successful visits are thought to be governed by the synergy between corolla tube and proboscis length and width (Brian, 1957). Pollinator guilds have been constructed based on the length of mouthparts and there is a general consensus that foraging efficiency is at a maximum when the proboscis length is shorter than, or equal to, corolla tube depth (Fontaine *et al.*, 2006; Diekötter *et al.*, 2010). Gómez *et al* (2008) echoed this and indicated that the positive associations between corolla tube length and nectar production, and corolla tube width and pollen production was responsible in driving the visitation of pollinators to *Erysimum mediohispanicum*.

The importance of pollen and nectar has been shown with regard to facilitation of further visits by pollinators (Cresswell, 1999). Pernal and Currie (2002) showed that honeybees were able to discriminate between pollen grains based on their lipid content, inferred through olfaction and grain size; small grains (optimal at 45μm) are lipid rich and starchless. Intraspecific discrimination has been suggested to increase foraging efficiency for the colony and also has metabolic implications for the individual pollinator. Optimal foraging theory stresses the need to maximise the net rate of energy intake to survive whilst foraging and scholars have indicated variability in nectar quality at the species, individual and flower level (Bosch, Retana and Cerdá, 1997; Potts *et al.*, 2003). Rewards (nectar and pollen) promote active learning and memorisation of visual and olfactory cues in pollinators, which in turn increases foraging efficiency (Duffield *et al.*, 1993).

Foraging efficiency, and thus pollination services, has diminished with the intensification of temperate grasslands and agricultural cultivation. Diekötter *et al* (2010) found that mass-flowering swards (crops) lead to a decline in the numbers of specialised and rare species of short-tongued bumble bees. Plant trait-based management of the agricultural landscape could instil greater bee diversity through an increase in plant functional diversity, however, the empirical research is inadequate for rigorously predicting pollination services. From this section, it could be ascertained that the indices derived from the Functional Diversity Hypothesis would play a significant role. The substantial role that native bee communities play in supporting pollination services is recognised and therefore maintaining and/or

restoring functional diversity in floral resources to temperate grasslands would be an effective multifunctional management practice. However, there is also utility in applying the Biomass-Ratio Hypothesis to optimise the production of a single crop through engineering a temperate grassland to support the most efficient pollinators. These two management scenarios are contrasted and developed in the context of *Malus domestica* orchards in 5.4.5ci.

5.4.45bi. Example: Apple Orchards

Apple cultivars are dependent on cross-pollination through self-incompatibility (Broothaerts, Van Neram and Keulemans, 2004). Insect pollination of apple crops is believed to increase fruit set and shape, enhance fruit quality and reduce fruit drop (Joshi and Joshi, 2010; Abrol, 2011). Cox and Gala varieties are the dominant cultivars grown by British growers and insect pollination was found to increase economic yield by £11000 and £14000 per hectare respectively (Garratt *et al.*, 2014). Insect pollination was also shown to increase apple weight, sugar content and overall fruit set (by 8%) in Cox apples (Garratt *et al.*, 2014). Supplying additional floral resources through temperate grassland restoration has been suggested as an approach to increase crop productivity as bee species richness was found to be critical; each individual bee species accounted for a 0.8% increase in the proportion of fruit set (Mallinger and Gratton, 2015). Apple growers typically aim for a 10% increase in the proportion of fruit set and therefore the surrounding landscape needs to support approximately 12 bee species to increase economic yield. In this respect, a functional diversity approach to the management and restoration of temperate grasslands is necessary to deliver ample floral resources. However, there is also the potential to engineer a temperate grassland system to optimise apple pollination by the most efficient bee species.

The functional differences in pollination efficiency have been revealed for the managed honeybee and wild bee species. Honeybees were found to be the least efficient pollinators because they sidework, or rob, nectar from apple flowers without contacting and fecundating the stigma and have demonstrated poor performance in unfavourable weather conditions (Boyle and Philogène, 1983; Martins, Gonzalez and Lechowicz, 2015). Wild bee families (Andrenidae and

Helictidae) are suggested to compensate for this shortfall; research has typically focussed on the pollination efficiency of four bee genera; *Andrena, Bombus, Osmia,* and *Apis*; that has been defined according to abundance and foraging efficiency (Figure-5.4.6).

	Pollination Efficiency		**Management Options**	
Bee Genus	*Abundance*	*Foraging Efficiency*	*Optimisation*	*Functional Diversity*
Apis spp	High	Low	No	Yes
Bombus spp	Low	High	Yes	Yes
Andrena spp	High	Low	No	Yes
Osmia spp	Low	High	Yes	Yes

Figure-5.4.6. Summary of the pollination efficiency of domesticated and wild genera and management options focussing on optimisation of apple yield and multifunctionality.

Figure-5.4.6. highlights the importance of *Bombus* and *Osmia spp* in the pollination efficiency of apple flowers, as informed by Martins, Gozalez and Lechowics (2015). *Osmia* spp. were found to the greatest contributor to Mexican apple orchards, and pollination by *Osmia cornuta* in European apple orchards increased commercial fruit set by 24.4% and the bees showed visiting rates as high as 98% (Bosch and Vicens, 2000; Rios-Velasco *et al.*, 2014). *Osmia* spp. are suggested to be eighty times more efficient than the managed honeybee because they land directly upon the reproductive structures of the apple tree blossom (Winfree *et al.*, 2008). A functional approach to the conservation and/or restoration of these four bee genera would promote diversity in floral and nesting resources to increase the abundance of *Bombus* and *Osmia*. The availability of nest holes has been identified as the greatest limiting factor to *Osmia spp* (Kraemer, 2001). An optimisation approach, on the other hand, would aim to promote populations of the most efficient pollinators through the provision of suitable floral and nest resources.

Regardless of the management approach, the provision of adequate reward resources would be vital to maintain pollinator populations. Garratt *et al* (2014) suggested that apple flowering lasts circa 10 days and there are commonly periods of food scarcity prior to and following flowering. This depletion of resources may reduce the fecundity and recovery of bee populations in apple orchards. Rundlöf *et al* (2014) showed that planting *Trifolium pratense* increased the reproductive success of long-tongued bumblebees; with a 71% increase in numbers of males and five times increase in the number of queens in mass-flowering apple orchards. It is clear that the supply of adequate pollen and nectar resources is important but greater analysis of temperate grassland species' floral resources with apple flowers is required. Optimal cross-pollination of apple orchards can be achieved if the time of flowering of apple orchards and nearby temperate grasslands is asynchronous and if, whilst flowering, apple flowers remain the most attractive floral units in the landscape.

5.4.5c. Advancing Methods of Study

The statistical analyses employed in this chapter relied on causal relationships through subset and lasso regression. Lasso regression is heavily criticised for its lack of significance testing and integration of model-selection methods. Research is needed to integrate estimates of bias, standard error and confidence intervals for prediction, efficient and linear combinations of coefficients (Hesterberg *et al.*, 2008). Hesterberg *et al* (2008) highlighted the need to integrate model comparison criteria, such as Mallow's C_p, Akaike Information Criterion, Bayesian Information Criterion, cross-validation and empirical Bayes. With these advances, the great potential of lasso is suggested to evolve because it is unique in its ability to produce exact zero estimates (Dormann *et al.*, 2012). Lasso was employed in this chapter due to its ability to cope with a limited number of measurements. The research of Hesterberg *et al* (2008) and Dormann *et al* (2012) interprets the results of lasso regression with caution. Given an expansive and balanced dataset, stronger conclusions could be drawn from the North Wyke Farm Platform using a more comprehensive statistical approach. Subset and lasso regression have been effective in investigating the direct influences of ecosystem processes/services but

the indirect effects are neither tested nor quantified. Grace *et al* (2010) indicated a need for suitable methods and procedures to study ecological communities and reviewed their implementation in ecological research.

Structural equation modelling has risen in popularity in ecology because it translates data and hypotheses into graphical models, which Fan *et al* (2016) highlight as a major advantage of the method. Trait-based approaches have indicated that functional identity is more important than diversity in driving ecosystem processes in temperate grasslands. Leaf economic traits were found to weakly drive nitrification potential, instead nitrification was strongly associated with abiotic soil properties (pH, temperature and nitrogen availability) (Mouillot *et al.*, 2011).

Structural equation modelling is a combination of two statistical approaches: confirmatory factor analysis and path analysis. Confirmatory factor analysis estimates latent variables, which are defined as hypothesised variables for which there are no direct measurements, for example functional structure and composition, abiotic and biotic pressures, productivity, stability and resilience (Grace *et al.*, 2010). Latent variables can be divided into measurable constructs, such as community-weighted mean of plant height, and could specifically indicate a predictor variable. The confirmatory factor analysis has the capacity to estimate latent variables based on the correlated variation of the dataset and reduces data dimensionality whilst standardising the scale of multiple indicators (Byrne, 2010). The subsequent path analysis aims to find causal relationships among latent variables by creating or testing a hypothesised path diagram of many different models. These structural meta-models are thought to be promising for incorporating trade-offs, feedbacks and multi-trophic interactions in a more explicit mechanistic manner (Byrnes *et al.*, 2014). It is widely recognised that structural equation modelling is a powerful tool for analysis that possesses the capabilities of bridging the gap between ecological theory and real-time data. However, Fan *et al* (2016) pointed to misuses and ambiguity in the published literature, especially surrounding the justification of final model selection (only 6.8% of studies justified model selection). Data scarcity and accessibility is the biggest hindrance and this chapter can be criticised on the same grounds, especially the livestock data for the

North Wyke Farm Platform. Despite this, Figure-5.4.7. summarises a basic structural equation model underpinned by the Response-Effect Framework for the end ecosystem service of animal production, which could be employed in future analyses.

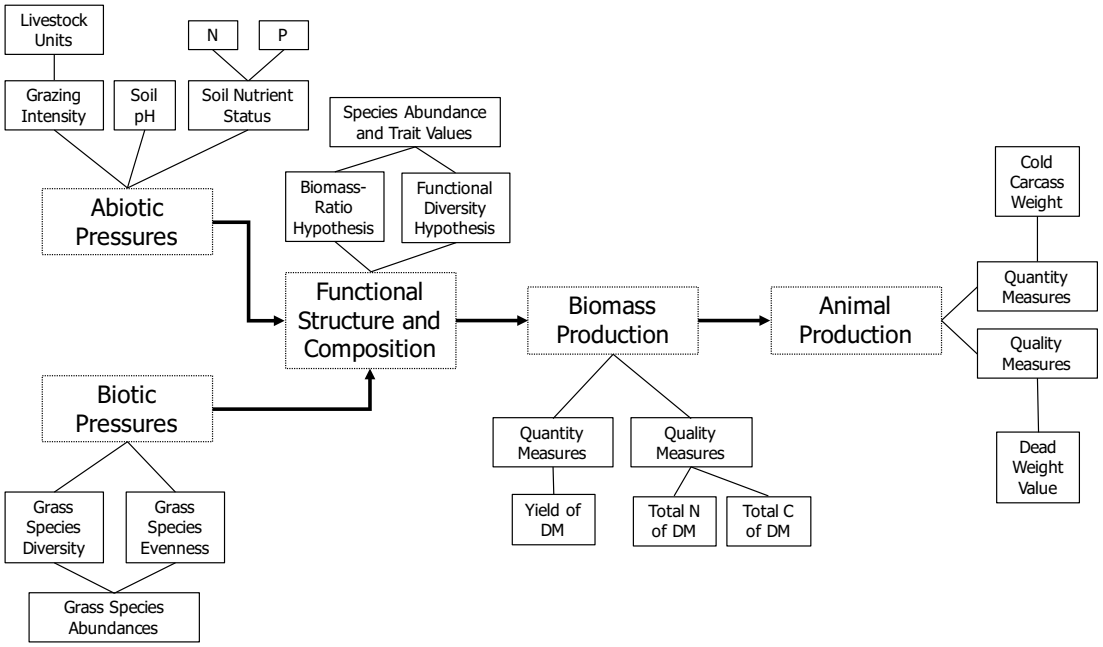

Figure-5.4.7. Basic structural equation model examining the associations between empirical measures (in rectangles) and theoretical constructs of the Response-Effect Framework (dotted outlines). The solid arrows indicate the conventional path of the Response-Effect Framework. Abbreviations: N, Nitrogen; P, Phosphorus; C, Carbon; DM, Dry Matter.

A Trait-Based Analysis of the Early Stages of a Grassland Restoration Experiment

6.1 Introduction

Two schools of thought exist when considering the assembly of a plant community: stochastic and deterministic processes. Stochastic assembly was conceptualised in Hubbell's unified neutral theory of biodiversity and biogeography, which assumes all plant species are ecologically equivalent and assemblages are random assortments of species (Hubbell, 2001). Hubbell received vast amounts of scrutiny from his peers due to the lack of experimental testing, but the neutral theory continues to be a rich source of quantitative null models to test non-random community assembly across spatial and temporal scales (Hubbell, 2006). Traditionally, community assembly has been viewed as a deterministic process in that species are subjected to a hierarchy of filters that dictate dispersal assembly rules, abiotic assembly rules, and biotic assembly rules. An actual community is, thus, a result of dispersal filtering of the regional pool followed by the abiotic and biotic filtering of the local species pool (Keddy, 1992; Götzenberger *et al.*, 2012). These successive filters and niche concepts were married in the Response-Effect Framework, which postulates a community is the result of non-random convergence (dispersal and abiotic filters) and divergence (biotic filter) processes. The utility of this framework in studying the resiliency and functionality of grassland ecosystems has been realised and could manifest as a framework to restore grasslands globally (Funk *et al.*, 2008; da Silveira Pontes *et al.*, 2015).

Germination and regeneration niches are of most significance to grassland restoration and have been conceptualised using plant functional traits. Seed traits are at the forefront of this research and their importance is continually stressed. These traits are assumed to capture germination and seedling establishment dynamics: rate of germination, germination success, seedling size, seedling growth and seedling survival (Nishizawa and Aarssen, 2014). Additionally, seed traits are postulated to exist on separate axis to whole plant and leaf traits, and are

independent of the CSR strategy informing primarily about regenerative strategies (Grime *et al.*, 1981; Laughlin, 2014b; Pierce *et al.*, 2014).

Morphological seed traits, such as seed mass, were found to be most commonly employed in publications since 1990 (Jiménez-Alfaro *et al.*, 2016). Seed mass has been suggested to reveal a remarkable spread within communities, around an order of five to six (Leishman, Westoby and Jurado, 1995). Seed mass has also been related to key germination and establishment processes, for example persistence and dormancy in soil seed banks and adaptations to environmental pressures. Small rounded seeds were found to consistently dominate the seed banks of temperate grasslands (Eriksson and Eriksson, 1997). Two classes of explanations delve into the longevity of these seed banks. The first assumes that seed mass is inherently related to seed number and dispersal distance. The production of copious amounts of small seeds facilitates a greater dispersal distance. This relationship is advantageous in environments where the dispersal filter and habitat filters are weak. Such has been observed in the soil seed banks of England (Rees, 1996). The second postulates that small rounded seeds escape predation by quick burial and incorporation into the soil profile (Chambers, Macmahon and Haefner, 1991).

Adaptations to disturbances have focussed on the scaling of seed mass with other germination and establishment traits. Larger seeds are thought to confer greater tolerance and plasticity to environmental pressures through three effect streams. The first assumes seed mass is positively related to seedling size and under shaded conditions larger seeded species are able to better compete for light and water resources (Westoby *et al.*, 1996). Increases in seed size were observed in heavily shaded British and European communities (Ryser, 1993). The second is grounded in the 'Cotyledon Functional Morphological' hypothesis that states larger seeds have a greater amount of total resources that can remain uncommitted and available to compensate for environmental perturbations, and therefore increases the likelihood of survival in unfavourable conditions, such as nutrient impoverishment (Jurado and Westoby, 1992; Westoby *et al.*, 1996). Finally, the slower metabolism of large seeded species (lower relative growth and respiration rates) enables longer survival under adverse conditions (Westoby *et al.*, 1996). Large seeds were found to have

longer cell cycles and larger genomes that demonstrated tolerance to low temperatures (Thompson, 1990). The benefits of greater seed mass, therefore, are concentrated on the better odds of withstanding environmental pressures and outcompeting smaller seeded species. Jiménez-Alfaro *et al* (2016) suggested such biophysical seed traits like seed mass would be strong predictors of germination and establishment processes.

The importance of germination traits was also expressed with regards to seed bank persistence (Saatkamp *et al.*, 2011). Germination traits, however, are vastly unquantified and studies. Currently, the TRY database covers 5.84% of plant species in respect to seed germination rate (germination efficiency). Germination traits (percentage germination and season of germination) have been utilised to define colonisation ability in a grassland restoration experiment (Pywell *et al.*, 2003). Colonisation ability was found to important in the early stages of ecological restoration and gradually decreased over time. Seedling relative growth rate was also highlighted as a plant trait significantly linked to colonisation ability and has been iterated by other scholars as an important trait determining plant strategy, relative competitiveness and establishment in restoration projects (Storkey, 2004; Cole and Lunt, 2005). For these reasons, plant traits describing germination and establishment are speculated to play a vital role in determining, and predicting, the occurrence of plant species (Olff *et al.*, 1994). In recent decades, models of community assembly have been developed to predict the species' abundances from plant traits.

6.1.1. A Modelling Approach of Trait-Based Community Assembly

The maximum entropy model (MaxEnt) was the first mathematical translation of trait-based community assembly (Shipley, Vile and Garnier, 2006). Community assembly was assumed to be a non-random process of species sorting through environmental filters underpinned by functional traits that determine a species' dispersal, growth, survival and reproduction. MaxEnt models predict relative species abundances based on four components defined in the model (Shipley, 2010).

1. Description of possible states (species) in which an entity can be found in a given system.
2. Description of prior probability distribution or density over these states.
3. A set of variable attributes.
4. Macroscopic constraints that represent average values of such attributes in the system.

The relative abundance of a species in a species pool is therefore a function of how closely its functional traits agree with the community-weighted means (Shipley, Vile and Garnier, 2006).

The empirical validity of the MaxEnt model has been shown in several studies and thought to have reignited modelling approaches of species abundances (Laughlin *et al.*, 2012). Non-random trait-based assembly was found to be dictated by interspecific differences in traits governing a species' immigration, survival and growth potential (Sonnier, Shipley and Navas, 2010). The application of MaxEnt to any ecosystem, number of species, traits and environmental variables has been praised, but testing in different environmental contexts is still lacking (da Silveira Pontes *et al.*, 2015). Sonnier, Shipley and Navas (2010) posited critical questions for the future of the MaxEnt model, one focussed on the number and identity of those functional traits that maximise predictive ability. Laughlin *et al* (2015) reported that between five to eight was optimal predicting the relative abundances of species from a regional pool. It has been postulated that expanding the suite of functional traits to include those that reflect regeneration, germination and establishment niches would increase model consistency.

6.1.2. Ecological Restoration of Temperate Grasslands

Much of the focus on the ecological restoration of grassland systems has centred on returning systems to a historical state (Figure-6.1.1.). In Europe, historical grassland states are defined by the abiotic and biotic levels of the pre-industrial revolution era, whereas the United States considers the period prior to European

settlement (Harris *et al.*, 2006). In doing so, current temperate grasslands can be viewed as novel (also known as emerging, or non-analog) ecosystems, which Hobbs *et al* (2006) defined as a system with an atypical species' structure and composition that is a result of deliberate or inadvertent human actions. Research across the globe documents the widespread intensification of temperate grasslands to enhance ecosystem processes and services, such as food production. These interventions are postulated to have steered grassland ecosystems along a novel trajectory. In New Zealand, European settlers changed the composition of tussocks from tall (*Chionochloa spp*) to short (*Festuca* and *Poa spp*) species through extensive pastoralism (frequent firing and heavy grazing) (Treskonova, 1991; Duncan, Webster and Jensen, 2001). With continued research, novelty has been reported across biomes and ecosystem characteristics – plant species diversity, ecosystem functions, resistance and resilience (Hobbs, Higgs and Harris, 2009). The quantification of novelty has also indicated a transient state (hybrid) (Hobbs *et al.*, 2006).

A hybrid system is defined as an amalgamation of historical and novel features. Hobbs *et al* (2009) suggested that the identity of these features is still debatable. Taking a trait-based perspective to ecosystem services, a plethora of intermediary scenarios exist but at one extreme the plant functional trait structure and composition is identical to historical states with a distinct ecosystem services profile, and vice-versa.

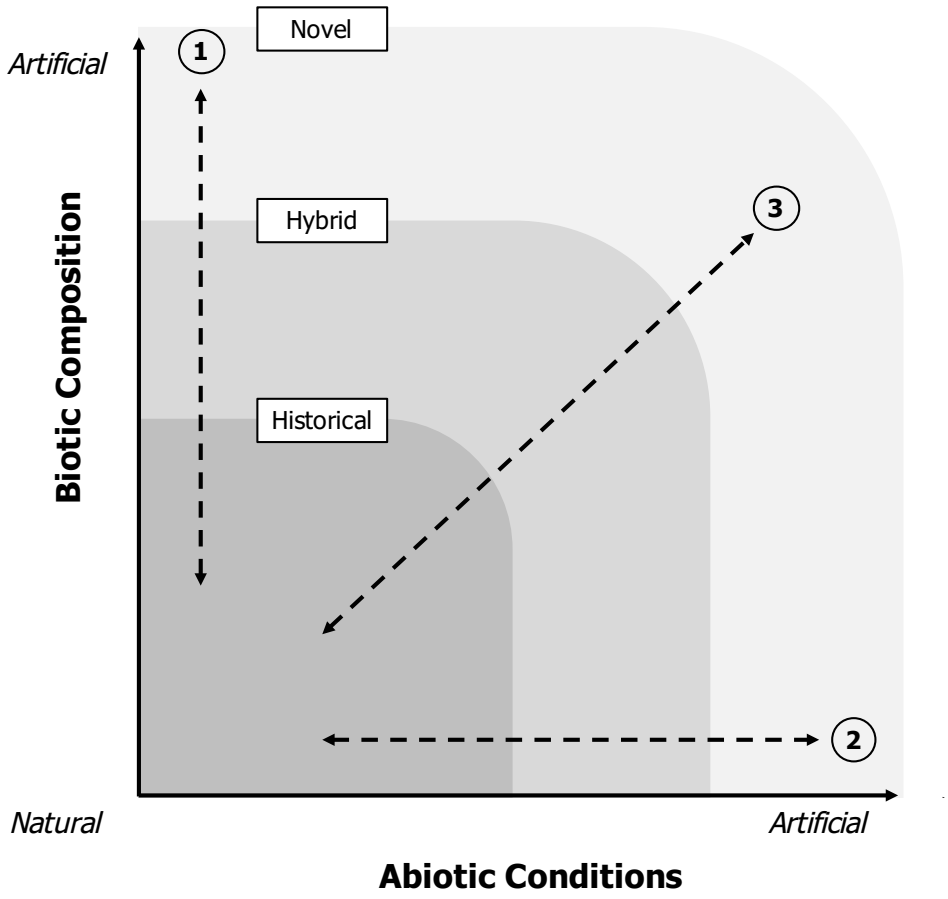

Figure-6.1.1. Three main ecosystem states that develop under differing levels of abiotic and biotic changes: historical, hybrid and novel. Dashed lines indicate trajectories that can be reversed by ecological restoration. Pathway one is driven by loss of native species' structure and composition and can reversed by overcoming the dispersal filter (6.1.2a.) and/or managing the biotic filter through removal of undesirable species (6.1.2c.). Pathway two coincides with change in abiotic conditions and ecological restoration through the manipulation of the abiotic filter (6.1.2b.). Pathway three is an amalgamation of the previous trajectories.

Despite the recognition of hybrid states, restoration projects are fixated on the complete rehabilitation of temperate grasslands. Focussing on concepts of nativity and naturalness are hoped to re-establish salutary relationships between people and ecosystem (Higgs, 2005). Ecologists are, therefore, tasked with defining historical states and exploring appropriate techniques to engineer a historical state. Historical states are determined by cultural, national and religious roots (aboriginal

knowledge and historical sources) and/or paleoecological records from natural archives and soil phytolith analyses (Hobbs, Higgs and Harris, 2009; Jackson and Hobbs, 2009; Evett, Dawson and Bartolome, 2013). Restoration practices are defined as active or passive. The latter is independent of human intervention and assumes natural processes will restore over time. Hindrances of passive restoration are widely recognised and functional ecology, and the Response-Effect Framework, has been hailed because it facilitates the visualisation and decomposition of these barriers; dispersal, abiotic and biotic filters (Figure-6.1.2). Funk *et al* (2008) stressed the utility and importance of these filters in designing active restoration projects. The coming sections review the ecological restoration methods, and the manipulations of each filter successively (6.1.2a. to 6.1.2c.) and evaluate the success of the methods in restoring a historical state (6.1.3.).

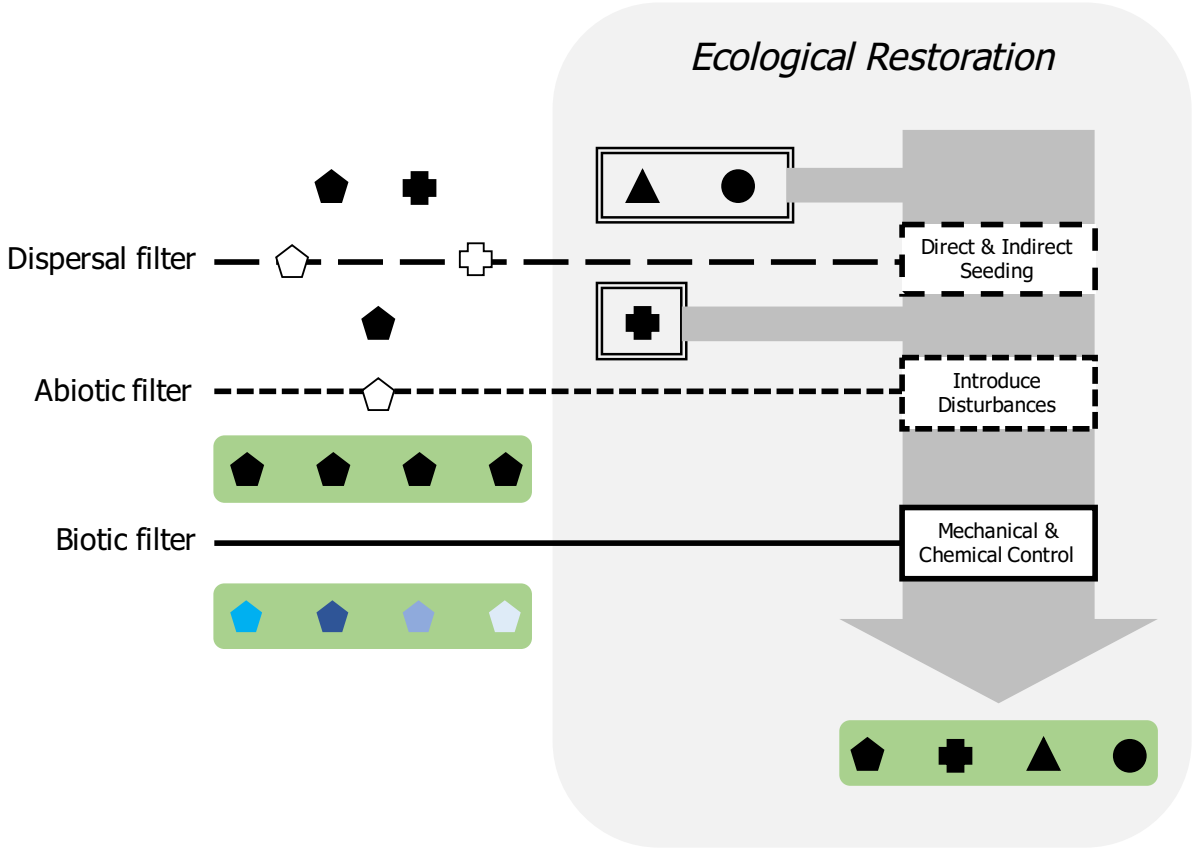

Figure-6.1.2. Conceptual model showing the filtering process as outlined in the Response-Effect Framework together with ecological restoration techniques that bypass these barriers.

6.1.2a. Overcoming the Dispersal Filter

The lack of propagule sources and dispersal barriers are major limiting factors for the ecological restoration of temperate grasslands (Hufford and Mazer, 2012). Fragmentation is cited as the largest barrier to natural recolonisation because with reducing patch size local extinction rates increase (Wallin, Svensson and Lönn, 2009). Local extinction is a result of accelerated inbreeding depression and increased genetic drift that lowers population diversity, fitness and adaptive potential, which forces the community into an extinction vortex (Aavik *et al.*, 2014). For ecological restoration, the reintroduction of propagules is vital to overcome the dispersal filter common in intensively managed landscapes (Gilbert, Gowing and Bullock, 2003).

Direct seeding methods (broadcast and drill) have seen variable success rates (10-40% establishment) (Jenkins, Gordon and Renda, 2004). Drill seeding has recorded higher germination and establishment rates due to the avoidance of granivorous predation but significant doubts exist in the naturalness of the method (Yurkonis, Wilsey, Moloney and van der Valk, 2010). The uniformity associated with drill seeding creates large contiguous spaces that act as physical niche gaps and are thought to increase the invasibility of the system, which is thought to influence the provisioning of ecosystem services (Yurkonis, Wilsey, Moloney, Drobney, *et al.*, 2010). Broadcast seeding, on the other hand, mimics natural dispersal and is more appropriate for large scale restoration projects (Sluis, 2002). Debates are ongoing about the specifics of direct seeding, such as mixture composition and origin.

6.1.2ai. Seed Origin and Mixture Composition

Contemporary restoration projects are focussed on restoring species-rich temperate grasslands under the Biodiversity-Ecosystem Function Hypothesis. Short term invasibility was found to decrease upon sowing a diverse seed mix (Falk *et al.*, 2013). Functional diversity is often disregarded in grassland restoration projects because of the uncertainty of defining functional trait groups and laborious task of gathering multi-trait data (Kimball *et al.*, 2014). Instead, research has fixated on defining the optimum species number in a mixture and the origin of those seeds.

Piper *et al* (2007) suggested eight plant species.

Seed origin is frequently explored with regards to the genetic diversity of mass-produced cultivar species. This issue has manifested given the widespread (approximately 70% of restoration projects) use of cultivar varieties, and thus the appropriateness of commercially available grassland seed mixes is questioned (Smith, Sher and Grant, 2007; Tischew *et al.*, 2011). Deducing the literature, scholars allude to plant functional traits in blaming the seed production industry for selecting for increase vigour of cultivar varieties by favouring domestication syndrome traits (Wilsey, 2010; Schröder and Prasse, 2013). These traits include lower seed dormancy, increased seed quality, faster germination, enhanced phytoperiod response, increased aboveground biomass, pest resistance and stress tolerance (Lulow, 2006; Klopf and Baer, 2011; Lambert, Baer and Gibson, 2011). The selection of domestication syndrome traits is suggested to increase restoration success via quicker establishment and production, as found by Lambert *et al* (2010) and the cultivar variety of *Andropogon geradii* possessing greater photosynthetic rate. The hypothetical advantages of cultivars are realised but in many cases cultivars fail to establishment via the loss of intraspecific variability, commonly referred to as local adaptation traits in Restoration Ecology (Fant *et al.*, 2008). Local adaptation traits are integral to the home site advantage hypothesis, which predicts locally adapted competitors will dominant the niche space through optimal resource capture and productivity (Hufford and Mazer, 2012). This draws parallels with the notion of abiotic filters proposed as part of the Response-Effect Framework and highlights key considerations for restoration managers when sourcing restoration seed mixtures. Economic constraints are the main cause of cultivar usage in temperate grassland projects.

The propagation of locally adapted seeds has received copious amounts of animosity from the seed production industry due to the lack of knowledge and funding (Peppin *et al.*, 2010). The propagation of a single ecotype requires collections of different populations and continual cross-pollination to maintain genetic diversity. Attaining an adequate number of collections to support production is a major issue when local extinction has already occurred. Many endemic

temperate grassland ecotypes are not commercially available because of this (Frischie and Rowe, 2012). For some restoration managers, the option exists to source local ecotypes independently via vacuum harvesting. Thirty individuals of the same ecotype is thought to imitate natural levels of intraspecific variation (Sinclair and Hobbs, 2009). However, various definition of locality has challenged the discrimination of ecotypes. Traditionally, geographic distance has been a classic measure of locality but it has been revealed as a poor indicator of the performance and survival of *Castilleja levisecta* in a restored prairie system (Lawrence and Kaye, 2011).

An extension of geographic locality has incorporated topographic, climate and edaphic data in defining zones of ecological similarity, ecoregions (Miller *et al.*, 2011). Ecoregions are presumed to capture sufficient genotypic variants to maintain natural diversity and secure long-term population viability (Wells *et al.*, 2003). The use of ecoregions to source propagules for direct seeding has been largely undocumented, instead, they have influenced indirect seeding techniques, such as endozoochory. Endozoochory relies on the targeted grazing of domesticated livestock on native grasslands and the subsequent deposition of native-seed containing dung on the area to be restored (Matías *et al.*, 2010). This indirect seeding method was found to intensify seed flow and influence species germination and establishment on donor sites (Traba, Levassor and Peco, 2003). The transferal of seed rich material has also manifested in the translocation of green hay from a local species-rich grassland to a receptor site (Stott, Dougher and Rew, 2010). This is assumed to provide a natural mix of locally occurring species that upon establishment produces a plant community that is spatially, floristically and functionally similar to the donor grassland (Desserud and Naeth, 2011).

6.1.2b. Manipulating the Abiotic Filter

6.1.2bi. Restoring Disturbance Regimes

Disturbances have long shaped the structure, composition and functioning of grassland ecosystems (Lake, 2013). The restoration of historical management practices is assumed to enhance species' nativity and support the delivery of

indigenous ecosystem services to a pre-European settlement level. Controlled and targeted disturbances modify the abiotic filter to select for native species and constructs a local species pool, a niche space and an ecosystem service profile characteristic of historical landscapes. In the Great Plains (United States), the reintroduction of aboriginal practices, such as prescribed firing, is postulated to increase wild food production, referred to as biocultural and ecogastronomic restoration (Nabhan, Walker and Moreno, 2010). Prairie chicken populations (*Tympanuchus spp*) are speculated to increase from targeted ecological restoration efforts and if exploited sustainably will restore traditional diets, reassert food sovereignty and provide additional income for impoverished rural communities. This is speculated to be further supported by the use of native keystone species like bison (*Bison bison*) to restore the landscape (Samson, Knopf and Ostlie, 2004). Much of literature on restoring disturbance regimes has focussed on the prescription of firing and grazing conducive to the germination and establishment of desirable plant species. Scholars have predominantly focussed on phenology to dictate the timing and intensity of the disturbance. The following sections will explore the research on prescribed firing and grazing through a plant traits lens.

6.1.2bii. Prescribed Firing of Temperate Grasslands

Burning of temperate grasslands in the United States was a common practice to maximise food production and maintain migration routes (Schultz and Crone, 1998). However, large-scale fire suppression and rapid agricultural cultivation during the era of European invasion degraded the landscape (Mullen, Springer and Kolb, 2006; Pyke, Brooks and D'Antonio, 2010). The formation of widespread cattle ranches eradicated local populations of digestible and palatable plant species, which were quickly replaced by introduced non-native productive grasses, such as *Cenchrus cilianis* (Tjelmeland, Fulbright and Lloyd-Reilley, 2008). In recent years, restoration projects have attempted to reconstruct the aboriginal firing regime with the premise that native populations will colonise – "build it and they will come" – based on their innate ability to avoid or tolerate firing.

Scholars have concentrated their efforts on the tolerance profiles of native plant species to prescribe an effective firing regime. Physiologically, fire tolerance is

represented by lower water content in vital tissues and seeds as heat conduction and mortality was found to be greatest in individuals with higher water content (Pyke, Brooks and D'Antonio, 2010). Carpological tolerance measures include greater maternal provisioning that increases seed quality via thicker seed coats. A thicker coat is typically associated with greater hardiness and viability as found for the Great Plains native of *Nassella pulchra* (Dyer, 2002). Critics argue that undesirable species can also possess fire adaption traits and readily recolonise the burned area; *Imperate cylindrical* (congongrass) was reported to rapidly regrow from an extensive rhizome system in response to aboveground biomass removal (O'dea, 2007; Holzmueller and Jose, 2010). Additionally, scholars have highlighted that shifting the seasonality of firing can filter undesirable species based on their phenology (Moyes, Witter and Gamon, 2005; Legge *et al.*, 2011).

Research has examined the effects of firing in spring, summer and autumn on the removal and recovery of a plethora of different plant species. In essence, early (spring) or late (autumn) season fires are recommended to avoid soil desiccation and seed mortality with summer firing (Copeland, Sluis and Howe, 2002). Spring burns were found to reduce seed viability by 96% in the non-native grass *Bromus diandrus* (great brome), reduce *Salix spp* vigour, and increase native grass and forb recruitment and establishment (Quinlan, Dale and Gates, 2003; Moyes, Witter and Gamon, 2005; Tix and Charvat, 2005; Bouressa *et al.*, 2010). In the United States, efforts to compile a database (Fire Effects Information System) documenting the responses of plant species to fire are ongoing and has resulted impacted the timing of prescribed firing. Pyke *et al* (2010) implemented the recommendations of the Fire Effects Information System to burn one or two months before the typical wildfire season to hinder the seed production of annual non-native species. Deviation from the aboriginal fire regime is becoming more common in ecological restoration and increasing the intensity of burning has arisen to manipulate the filtering of undesirable species.

Intensity of firing is defined here by the frequency. More frequent fires were found to eliminate undesirable species from the seed bank, reduce woody encroachment and ultimately stimulate native grass species colonisation (Cione, Padgett and Allen,

2002; Copeland, Sluis and Howe, 2002). Frequent firing continually removes standing and dead litter biomass, which alleviates depressed resource and germination niches. The increase in light penetration to the soil surface generates temperature fluctuation that break seed dormancy (Maret and Wilson, 2005). Additionally, frequent fires have been associated with increased nutrient volatisation and surface run-off leading to reduction in soil nutrient availability (Pyke, Brooks and D'Antonio, 2010). Increasing the intensity of firing, therefore, facilitates the germination and establishment of slower-growing, and commonly desirable, species by modifying the abiotic filter to redirect the trajectory of temperate grassland towards a more desirable state.

6.1.2biii. Targeted Grazing of Temperate Grasslands

During the agricultural intensification of temperate Europe, different regions were simultaneously isolated from native herbivores and grazed using domestic livestock. This is postulated to have diversified European grasslands. Species-rich semi-natural grasslands evolved from livestock herbivory during this era and since agricultural abandonment, the plant species assemblages have transformed into an undesirable state; presence of environmental weeds and woody species (Hejcman *et al.*, 2013). The reinstatement of low intensity rotational grazing with livestock is thought to restore a native plant community. Kleppel *et al* (2011) suggested native flora richness increased when a non-native dominated grassland system was grazed 20% of the time. The use of periodic short/moderate intensity grazing has been supported in other research (Whalley, 2005; Li *et al.*, 2008). Targeted grazing has been outlined as an effective option for temperate Europe, especially since the grassland systems are largely fire-intolerant (Kleppel *et al.*, 2011). Consequently, the reintroduction of free ranging large herbivores (cattle, horses and sheep) to restore temperate grassland systems is assumed to suffice (Yelenik and Levine, 2010). Theoretically, targeted grazing is speculated to modify the abiotic filter to be conducive for desirable species' germination and establishment; undesirable plant species are the most palatable and thus selectively browsed. The strength and selectivity of this abiotic filter is defined by grazer species, timing and intensity.

Bartolome (2004) found that grazing shifted an annual species dominated system into a perennial, and more desirable, grazing-tolerant community. Early spring grazing was found to beneficial for mature *N.pulchra* individuals but hindered the establishment of seedlings (Dyer, 2003). Moreover, *Bison bison* and *Boa taurus* were found to preferentially graze native C4 grasses, which caused gradual invasion of non-native C3 over the study period (Jackson, Paine and Woodis, 2010). On the contrary, the exploitative grass species (*Molinia caerulea*) was reported to be more effectively controlled by cattle than sheep in restoring *Calluna vulgaris* heathlands in the United Kingdom (Critchley *et al.*, 2008). These studies stress the importance of the filtering processes tied to herbivore and plant species. Due to scholarly inadequacies, a database documenting the responses of individual plant species to herbivory (herbivore species, timing and intensity) is lacking. A combined functional trait analysis of herbivore and plant species could shed light on herbivore guilds and their preferred suite of plant functional traits. This effort would facilitate the selection of herbivore species and grazing intensity to actively manipulate the filtering of desirable plant species and positively impact ecological restoration practices.

6.1.2biv. Reducing Edaphic Pressures

Decades of fertiliser use, and continual cultivation have deposited huge amounts of anthropogenically-derived nitrogen and exhausted soil moisture. These added edaphic pressures have selected early-successional plant species, typically weedy annual species that adopt the exploitative strategy and reduce native species diversity in restored grasslands (Gilbert, Gowing and Bullock, 2003; Rowe, Brown and Paschke, 2009). Lowering plant available nitrogen has thus become the main focus in grassland restoration projects under the assumption of restoring a natural abiotic filter.

Within the literature there are three main ways in which plant available nitrogen is reduced: carbon addition, mulching and ephemeral cover crops. The latter utilises temporary crops to reduce plant available nitrogen whilst reducing soil erosion, increasing water infiltration and suppressing weedy plant species invasion (Espeland and Perkins, 2013). Rotations of soybean-corn-soybean followed by the seeding of

native grassland species have been used in restoration projects but the efficacy of sterile annual hybrid grasses has been suggested (Morris and Schupp, 2009). Carbon addition and mulching are known as reverse fertilisation techniques (Averett *et al.*, 2004). The introduction of a carbon source (mulch, sucrose, sawdust, woodchips) stimulates a shift in the microbial food web, favouring carbon-limited microbe populations, that promotes nitrogen immobilisation and the reduction in plant available nitrogen (Bleier and Jackson, 2007; Doll *et al.*, 2011). Corbin & D'Antonio (2004) and Bleier and Jackson (2007) suggest that sawdust addition results in the highest level microbial biomass, nitrogen immobilisation and reduction in plant available nitrogen. This reduction has been shown to increase the germination rate of dry seeded plant, grass species and slower-growing grassland plants (Banerjee, Gerhart and Glenn, 2006). Mulching differs in that it can protect the soil surface from erosion and soil water evaporation (Petersen, Roundy and Bryant, 2004). Carbon addition and mulching both produce temporary reductions in nitrogen levels that creates small windows of opportunity for grassland species to germinate. Rowe *et al* (2009) found that native plant species cover increased by 13.4% when plant available nitrogen was reduced.

6.1.2c. Managing the Biotic Filter

It is estimated that 25% of ecological restoration effort is solely dedicated to the removal of undesirable plant species, particularly those introduced by human interventions (Price and Weltzin, 2003; Rowe, 2010). Intensification of and improvement of grazing pastures was traditionally achieved through the introduction of productive non-native grass species to functionally restore the natural capital- ecosystem functioning (Sluis, 2002). The non-natives were assumed to improve ecosystem functioning through retaining soil moisture, structure and nitrogen and carbon pools (Firn, 2007; Klopf and Baer, 2011). As part of the United States Conservation Reserve Program, the establishment of non-native species was encouraged such that 2800 non-native plant species have been introduced. The greatest barrier for ecological restoration resides with those species that are naturalised – predicted to approximately three hundred species. *Cenchrus cilianis* (Buffelgrass) has been identified as a problematic naturalised environmental grass weed, which was introduced from Africa (Daehler and Goergen, 2005; Grice, 2006).

Until recently environmental weeds received little governmental and scientific attention. The major characteristic studied is competition dynamics underpinned by grassland species' life history strategies (annual vs perennial). Non-native environmental weeds are typically annuals possessing an early phenology that enables them to pre-empt resources and allocate them accordingly (Moyes, Witter and Gamon, 2005; Davies and Sheley, 2011). Non-natives were found to invest photosynthate resources in developing extensive deep taproots in the soil profile, which in turn lowers the nutrient and water availability before slowing developing native species can establish (Young *et al.*, 2009; Bonebrake *et al.*, 2011). *Centaurea maculose* (Spotted Knapweed) was found to adopt this annual strategy and secretes a phytotoxin (catechin) to hinder the germination and growth of native plant species (Perry *et al.*, 2005; Macdonald, Scull and Abella, 2007).

The capacity of non-native species to manipulate various environmental gradients has deemed them effective ecosystem engineers. *Bromus tectorum* (Cheatgrass) was found to change the composition of the soil microflora especially the arbuscular mycorrhizal fungi from a mutualistic species to parasitic and saprophytic forms (Rowe, Brown and Paschke, 2009). Additionally, cheatgrass has been reported to increase the frequency of fires in the United States via increased fuel load. The introduction of a new firing regimes threatens the native species that lack traits that aid post-fire recovery, thus perpetuating the expansion of non-native annual species to adjacent areas (Steers and Allen, 2010; Davies and Sheley, 2011). The formation of this new fire regime decreases native species richness and shifts the successional trajectory of a grassland system towards a novel structure and composition that supports a different ecosystem services' repertoire (Hobbs, Higgs and Harris, 2009).

The ecological restoration of novel temperate grasslands has concentrated on eradicating non-native and undesirable species to reverse or adjust the ecological trajectory (Hamman and Hawkes, 2013). Restoration projects have focussed on targeted physical and chemical disturbances.

6.1.2ci. Physical and Chemical Eradication

The introduction of physical and chemical disturbances to remove undesirable plant species has been a common theme throughout the Anthropocene. Hand weeding being the most primitive method. Research has indicated the restoration potential of hand pulling at small scales demonstrated in the removal of *Corpobrotus spp* in Andalucia, Spain, and restoration of the native forb (*Ambrosia pumila)* California, United States (Andreu *et al.*, 2010; Hasselquist, Hasselquist and Rogers, 2013). Despite its limitations (labour intensive and expensive) hand weeding is effective in selectively removing undesirable plant species (Kimball *et al.*, 2014).

Mechanical removal often utilises farmland cultivation methods and machines. The use of root ploughs is suggested to destroy the natural stratification of the soil and alter organic matter retention, as well as destroying undesirable plant architecture. Total soil carbon, microbial biomass and respiration levels fall and with the change in aboveground biomass the biological and physical legacies of the undesirable system are eliminated, thus forming regeneration niches (Potthoff *et al.*, 2005). Sher *et al* (2008) demonstrated this notion of competitive release through the mechanical removal of *Tamarix* spp and the proceeding germination of *B.tectorum*. Other pedocentric approaches have included topsoil removal, found to promote the recolonisation of target plant species and progression towards a native sward structure (Olsson and Ödman, 2014). Topsoil removal is an effective approach when the undesirable plant species dominate and persist in the soil seed bank.

Mechanical mowing is widely implemented in grassland restoration projects. It adheres to the same rationale as grazing and burning, of removing aboveground biomass, opening niche space, creating spatial heterogeneity that supports the germination of slower growing native grassland species (Dodd and Burns, 2013). Mowing has been shown to increase the abundance of native grassland species, at the detriment of trees, shrubs and competitive undesirable species, and increase grassland species diversity - particularly forbs (Ilmarinen *et al.*, 2009; Gonzales and Clements, 2010; Klimkowska *et al.*, 2010). Mowing encourages forb seedlings, specifically broad-leaved, to reach a life form that will support its survival in successive growing seasons, as found by Williams *et al* (2007) and Prach *et al*

(2012). Continuous mowing is necessary for the longevity of the restored grassland because mowing is suggested to control undesirable plant species in the seed bank as well as lowering soil nutrient concentrations by disputing the abundance and activity of soil decomposers (Ilmarinen *et al.*, 2009; Lambrecht and D'Amore, 2010; Marushia and Allen, 2011).

The utilisation of on-farm practices to restore grassland plant communities extends further to chemical control methods. Herbicide application is a common practice in contemporary agriculture, and given the intimate relationship with grassland restoration projects, the method couldn't be more apt. Selective measures, such as spot-treatments of glyphosate, have been shown to reduce the abundance of non-native grass species and promote pioneering native forb species (Hoeffner, 2010). Bracken (*Pteridium aquillium)* is an example of an undesirable fern species in the United Kingdom controlled using herbicides, such as Asulam, to restore desirable species to pastureland (Ghorbani *et al.*, 2007; Cox *et al.*, 2008). Grass-specific herbicides, such as Fusilade II and Tebuthiuran, are cited in the literature to reduce non-native grasses (Buffelgrass) and forbs, whilst increasing the cover of desirable grasses (Tjelmeland, Fulbright and Lloyd-Reilley, 2008; Steers and Allen, 2010). Some herbicides, specifically post-emergent, are shown to minimise the reinvasion of non-native species (Cione, Padgett and Allen, 2002; Flory, 2010). However, some scholars are particularly critical of herbicides given their discourse in agricultural science; herbicide treatment resulted in persistent populations for 34 years that required further management strategies (Wilson and Pärtel, 2003). Follow-up applications or coupling with pre-treatments (disking or kinetin) may improve the efficacy of herbicide treatment (Annen, 2010). However in some circumstances, as with wheatgrass, emergence was noted, even with 10 years of glyphosate treatment (Ambrose and Wilson, 2003). Research has indicated combinational treatments with other disturbances, such as grazing or firing. Stanley (2011) reports the most effective method to control non-native perennial grass and forb species was sethoxydium-burning-glyphosate. Combinations with grazing are also endorsed (Girard-Cartier and Kleppel, 2015).

6.1.3. Progression Towards Trait-Based Restoration of Temperate Grasslands

Despite great efforts to restore historical states to grassland systems, much of the literature reports inconsistencies in the short- and long-term success. Moreover, there is a general underreporting of failed projects and research (Maron *et al.*, 2012). The germination and establishment failures of historically-relevant species has been the greatest barrier to grassland restoration projects due to the lack of complete control of filters (dispersal, abiotic and biotic). Purely managing the biotic filter was found to result in the re-establishment of undesirable species from the seed bank or rhizome bud bank (Gonzales and Clements, 2010). Even when native plant species are introduced via direct or indirect seeding methods, the lack of germination niche spaces due to the legacy effects of intensification hinder native plant species. Coordinated manipulations of the dispersal, abiotic and biotic filters are, therefore, needed in the early stages of ecological restoration to influence the progression to a historical state. However, scholars are continually questioning the validity of these targets, especially in the face of climate change, and have urged a shift away from taxon-specific targets like the richness and abundance of indicator, keystone and valued species (Block *et al.*, 2001; Babin-Fenske and Anand, 2010). The emphasis on taxon-specific approaches has resulted in many examples of shortcomings, particularly in the assessment of ecosystem functioning and service provision (Gibbons *et al.*, 2009). Functional targets are suggested to resolve the simplicity of taxon-specific measures and accurately capture elements of community stability, invasibility and functionality (Young *et al.*, 2009).

6.1.4. Scope of the Chapter

Functional targets force the re-evaluation of ecological restoration practices and posits questions about the validity of current techniques. Depending on the goal, a temperate grassland system could be designed to optimise the delivery of a single ecosystem service or support multifunctionality irrespective of nativity and naturalness. However, a better understanding of community (re)assembly processes and the hindrances of temperate grassland restoration is needed beforehand. This chapter proposed and examined three specific hypotheses to shed light on these gaps in trait-based restoration of temperate grasslands.

6.1.4a. Hypothesis 1: Modelling of Community Assembly

Sonnier, Shipley and Navas (2010) urged studies to investigate the dimensionality and identity of plant traits that optimise the prediction of non-random community assembly processes. Seed and germination traits will feature in the optimal maximum entropy model together with mature plant traits.

6.1.4b. Hypothesis 2: Seed/Germination Traits and Abiotic/Biotic Pressures

The germination and establishment success of a plant species has been related to the scaling of seed mass with other traits. For example, environmental weeds have a quick germination response to high soil nutrient status. It was, therefore, hypothesised that the seed/germination trait profile of dominant species will reflect this strategy, specifically typical post-agricultural soils (high soil nutrient status and presence of weedy species in the seed bank). Furthermore, it is expected that a restoration mixture resembling local temperate grasslands will have a trait profile conducive of late-successional plant species (larger seeds, slower seedling relative growth rate).

6.1.4c. Hypothesis 3: Restoration Targets and Management Options

Coordinated efforts to manipulate dispersal, abiotic and biotic filters are lacking in ecological restoration research. Thus, questions remain on about which, and to what level should abiotic and biotic condition be changed to achieve traditional vegetation and functional targets. This chapter hypothesises that soils with a strong agricultural legacy (high soil nutrient status and weedy seed bank) will greatly hinder the progression towards both vegetation and functional targets.

The aim of this chapter was to reveal the trait-based assembly of temperate grassland communities and understand the impacts of abiotic and biotic factors during seed germination and establishment, and their effects on success in the early stages of temperate grassland restoration experiment. Through an experimental design, this should increase the understanding of the germination and establishment phases early in restoration projects (first eight weeks) and inform the

future management of temperate grasslands prior to direct seeding. Increased knowledge allows restoration and land managers to make informed decisions on the validity of certain ecological restoration techniques to achieve targets.

6.2. Materials and Methods

6.2.1. Experimental Design

The experiment was conducted in the Dutch Light Frame Plot 11 located within the experimental fields of Warwick Crop Centre, University of Warwick, from June to August 2014. A randomised design was used to test the treatments of soil nutrient status, seedbank composition and grassland seed mixture. Two nutrient status treatments (post-agriculture and restored soil conditions), presence of grass or broad-leaved weed species, and three grassland seed mixtures provided a total of twenty-four combinations, each of which was replicated five times. Each of the 120 tubular microcosms in this experiment had the dimensions of 0.225 X 0.08m and was filled with treatment-specific topsoil loam (Figure-6.2.1.). All pots were free-draining and independent.

Figure-6.2.1: Microcosms in the Dutch Light Frame Plot 11.

6.2.2 Treatment Structure

There were two soil nutrient statuses. The two conditions were constructed to differ in nitrogen, phosphorus and potassium to investigate the impacts of abiotic agricultural legacies on the patterning of seed and germination traits (Hypothesis 2)

and effects on the progression towards restoration targets (Hypothesis 3). Through communications with Natural England, estimates of the appropriate levels for these treatments were defined (Table-6.2.1.).

	Post-Agriculture	**Application Rate (kg/ha)**	**Restored Soil Conditions**	**Application Rate (kg/ha)**
Soil Nitrogen Supply Index	1	815	0	185
Olsen's Phosphorus	2	2440	1	1040
Exchangeable Potassium	2-	1840	2+	2675

Table-6.2.1: indices of nitrogen, phosphorus and potassium used to create the post-agriculture and restored soil conditions.

Using these estimates, and the baseline recordings of the manufactured top soil loam (brand "Erin"), inorganic fertilisers were applied the soil to increase the levels of nitrogen (ammonium nitrate- 27%N), phosphorus (triple superphosphate – 44%P) and potassium (sulphate of potash – 50%K)- application rates according to baseline conditions are shown in Table-6.2.1. This was achieved through the addition of weighed ammonium nitrate, sulphate of potash and triple superphosphate to specific volumes of topsoil in a cement mixer. This was executed in a batch process, mixing seventy-five litres of soil at once.

For each soil nutrient status, there were four different seedbank compositions devised to investigate the impacts of biotic agricultural legacies on the patterning of seed and germination traits (Hypothesis 2) and effects on the progression towards restoration targets (Hypothesis 3). The weed species included in each of these treatments are summarised in Table-6.2.2. The selection of species was based on communication with Peter Lutman, Stephen Moss, Jonathan Storkey and Paul Neve,

together with Lutman *et al* (2009). This paper suggested that the most abundant weeds in arable weeds in southern England were *Alopecurus myosuroides*, *Poa annua, Galium aparine* and *Veronica persica*. Additionally, a seedbank density of approximately 7000 seeds/m^2 was gleaned from these discussions. The weed species of each condition were given equal weighting, and therefore equal quantities in the seedbank. The counted seeds were added to each soil nutrient status treatment at the time of mixing. The seeds were sourced from Herbiseed.

Seedbank Composition	**Species**
Grass Species	*Alopecurus myosuroides, Bromus sterilis, Avena fatua, Lolium multiflorum, Poa annua.*
Broadleaf Species	*Capsella bursa-pastoris, Chenopodium album, Galium aparine, Papaver rhoeas, Polygonum aviculare, Senecio vulgaris, Sinapis arvensis, Stellaria media, Veronica persica, Viola arvensis.*

Table-6.2.2: The seedbank compositions and the weedy species that constitute them.

The constructed soil conditions (soil nutrient status and seedbank composition) were translocated and the microcosms filled. Each microcosm was then sown with one of three grassland seed mixtures (shown in Table-6.2.3.). Two of these seed mixtures were selected from the range of commercially available environmental scheme seed mixtures available from Cotswold Grass Seeds Direct. The "Local" seed mixture was defined by Natural England (Nick Woodward) and resembled a grassland restoration project that was undertaken in the West Midlands in 2014. Each microcosm was hand-sewn following the treatment structure and to the quantities of: Local (2g), ResA (2g) and ResB (1.2g). Species abundances (%) were measured and recorded for each microcosm at intervals of one, two, four and six weeks after sowing.

Restoration Seed Mixture	**Species (% in mixture)**
Local	*Agrostis capillaris (5%), Cynosurus cristatus (10%), Anthoxanthum odoratum (5%), Briza media (10%), Festuca rubra (20%), Festuca pratensis (25%), Lotus corniculatus (1%), Trifolium pratense (2%), Lathryus pratensis (1%), Leucanthemum vulgare (2%), Galium verum (2%), Prunella vulgaris (2%), Leontodon hispidus (1%), Rhinanthus minor (1%), Centaurea nigra (2%), Leontondon autumnalis (1%), Ranunculus acris (2%), Vicia cracca (1%), Plantago lanceolata (2%), Filipendula ulmaria (1%), Malva moschata (1%), Silene dioica (2%).*
ResA (Floristically Enhanced Mix)	*Agrostis capillaris (5%), Cynosurus cristatus (10%), Phleum pratense (5%), Festuca rubra (25%), Festuca brevipila (21%), Poa pratensis (25%), Knautia arvensis (1%), Centaurea nigra (1%), Ranunculus acris (1%), Prunella vulgaris (1%), Achillea millefolium (1%), Leucanthemum vulgare (1%), Galium verum (1%), Malva moschata (1%), Daucus carota (1%).*
ResB (The Operation Pollinator Mix)	*Trifolium pratense (56%), Trifolium hybridum (20%), Onobrychis spp (20%), Lotus corniculatus (2%),*

	Centaurea nigra (1%), Malva moschata (1%).

Table-6.2.3: the grassland restoration seed mixtures and the species components.

6.2.3. Germination, Regeneration and Establishment Plant Traits

Data on plant traits were gathered for the fifty-six species used in this chapter. Continuous trait values were gleaned from the published literature and databases for three additional traits (seedling relative growth rate, 2C DNA content and t_{50}) (Table-6.2.4.). To ensure appropriate scales and techniques, research was accumulated based on the methods outlined in Grime *et al (*1981). These new traits, together with seed mass, were used to represent germination, regeneration and establishment niches.

2C DNA content and t_{50} have been suggested to reflect temporal niche differentiation (Grime *et al.*, 1981). For 2C DNA content, weights per nucleus were based on Feulgen staining and microdensitometry of root-tip preparation. Root tips are fixed, hydrolysed, stained with Schiffs reagent, squashed and mounted on glass DePex. The density of stain in the early prophase nuclei was estimated using Vickers M86 scanning microdensitometer and measured in picograms. T_{50} is the number of days following imbibition of air-dried seeds required for half of the final percentage germination to be attained (t_{50}); typically seeds ripened by dry storage in the laboratory, were incubated on moist Whatman No.1 filter paper in Petri dishes in the light. Species with high nuclear DNA content and low t_{50} values are suggested to mirror the evolution of rapidly-growing ephemeral species, such as arable weeds and annual crops. Both traits have also been important in predicting and interpreting seasonal patterns of shoot development (timing and rate); nuclear DNA positively and t_{50} negatively (Grime, Hodgson and Hunt, 2007). A more direct measure of shoot development and growth rate was quantified as seedling relative growth rate. This is measured as the change in plant mass relative to whole plant mass per day (g/g/day). Grime *et al* (1981) measured seedling relative growth rate between two and five weeks after germination. Higher seedling relative growth rates have been associated with ruderal plant species in productive habitats (Grime,

Hodgson and Hunt, 2007). These three traits together with seed mass were found to be independent (highest R^2 recorded was 0.141) and therefore were used to calculate the functional diversity indices.

Trait	**Sources**
Seedling Relative Growth Rate (g/g/day)	(Wilson and Johnston, 1969; Poorter and Remeks, 1990; Seel, Parsons and Press, 1993; Fitter and Peat, 1994; Almeida-Cortez and Shipley, 2002; Hofmann and Isselstein, 2004; Storkey, 2004, 2006; Mwangi *et al.*, 2007).
2C DNA Content (pg)	(Bainard *et al.*, 2012; Pustahija *et al.*, 2013; Royal Botanical Gardens Kew, 2018).
Germination Rate/T_{50} (number of days)	(Grime *et al.*, 1981; Gibson and Newman, 2001; Gundel *et al.*, 2006; van Assche and Vandelook, 2006; Grime, Hodgson and Hunt, 2007; Jabran *et al.*, 2010; Toorop *et al.*, 2012; Délye *et al.*, 2013).
Seed Mass (mg)	(Kattge *et al.*, 2011).

Table-6.2.4. The sources of published literature for seed and germination traits employed in this chapter.

6.2.4. Statistical Analyses

6.2.4a. Effects of Manipulating the Abiotic and Biotic Filters on the Early Stages of Grassland Restoration

A five-way analysis of variance was conducted to assess the impacts of nutrient status, seedbank composition, restoration mixture and time on germination and establishment traits. Community-weighted means were calculated to represent the

associated germination and establishment niche spaces. Significant interactions were analysed by decomposing them into series of lower order effects that were examined with simple effects tests. Post hoc analyses of these simple main effects were conducted using Tukey's HSD. The assumptions of heteroscedasticity and normality were checked using diagnostic plots.

6.2.4b. Modelling the Early Stages of Grassland Restoration

The MaxEnt model was implemented to test the number of plant traits that predict the relative abundances of each species in a microcosm. Macroscopic constraints of community-weighted means were calculated for ten plant traits. Significance tests were performed using the permutation test (function *maxent.test*, *FD* package) with 1000 permutations. The null hypothesis was that traits are independent of relative abundances, and therefore indicate random community assembly. In total, 1055 MaxEnt models and permutation tests were performed to examine the different number (one to ten) and identity of plant traits that best predict the species abundances in all microcosms. One-way analysis of variance and Tukey's HSD were executed to determine the effect the number of traits has on the r^2 values of the MaxEnt models.

6.2.4c. Examining the Progression Towards Mature Restoration Targets

Grassland restoration targets were defined according to the species proportions of the local, ResA and ResB seed mixtures. The restoration targets included: vegetation structure and composition (species' relative proportions), and functional structure and composition (FRic, FEve and FDiv). The Bray-Curtis measure was used to quantify dissimilarities between species' abundances in the microcosms and their associated seed mixture. Euclidean distance was used to calculate dissimilarities in functional structure and composition. The effect of soil nutrient status, seedbank composition and time on the dissimilarities in vegetation and functional structure and composition were investigated using a four-way analysis of variance. The main effects were explored and decomposed from significant interaction effects and Tukey's HSD tests were implemented.

6.3. Results

6.3.1. Effects of Abiotic and Biotic Filters on Germination and Establishment Traits

The five-way analysis of variance yielded significant interactions at the three- (2C DNA content) and four-factor levels (seed mass, seedling relative growth rate and germination rate). These interactions were decomposed to analyse the main effects of nutrient status, weedy broadleaf species, weedy grass species and restoration mix. These results are detailed in 6.3.1a to 1c.

6.3.1a. Nutrient Status

The results of the main effects of soil nutrient status (Table-6.3.2.) demonstrate that there were significant impacts on the developing communities' seed and germination trait structure. Post agricultural soils were found to generally promote the rapid germination of small seeded species with low nuclear DNA content. Additionally, the dominant establishing seedlings were found to have increased relative growth rates, particularly in the absence of weedy broadleaf species. In contrast, their presence in the soil seed bank, on average, reduced the community relative growth rate by half.

Model$_{[df]}$	Seed Mass	Seedling Relative Growth Rate	Germination Rate	2C DNA Content
Nutrient Status$_{[1,279]}$	*72.20 ****	*0.02*	6.74 **	219.11 ***
Weedy Broadleaf Species$_{[1,279]}$	*7.68 ***	*28.88 ****	21.61 ***	46.94 ***
Weedy Grass Species$_{[1,279]}$	*46.17 ****	*0.17*	13.86 ***	5.67 *
Restoration Mix$_{[2,279]}$	*39.09 ****	*17.75 ****	1.33	51.66 ***
Time$_{[3,279]}$	*0.43*	*0.04*	3.30 *	0.03
Nutrient x Broadleaf$_{[1,279]}$	*23.64 ****	*13.40 ****	1.19	28.37 ***
Nutrient x Grass$_{[1,279]}$	*130.04 ****	*97.94 ****	40.80 ***	94.72 ***
Broadleaf x Grass$_{[1,279]}$	*22.23 ****	*24.44 ****	64.12 ***	208.00 ***
Nutrient x RestMix$_{[2,279]}$	*33.99 ****	*16.00 ****	21.91 ***	18.46 ***
Broadleaf x RestMix$_{[2,279]}$	*26.94 ****	*20.78 ****	19.56 ***	21.08 ***
Grass x RestMix$_{[2,279]}$	*9.27 ****	*2.41*	1.58	0.72
Time x Nutrient$_{[3,279]}$	*0.54*	*0.05*	3.18 *	0.25
Time x Broadleaf$_{[3,279]}$	*0.19*	*0.04*	0.62	0.19
Grass x Time$_{[3,279]}$	*0.41*	*0.54*	1.71	0.61
Time x RestMix$_{[6,279]}$	*0.12*	*0.01*	1.12	0.26
Nutrient x Broadleaf x Grass$_{[1,279]}$	*481.52 ****	*47.80 ****	1.41	154.27 ***
Nutrient x Broadleaf x RestMix$_{[2,279]}$	*136.58 ****	*12.00 ****	5.19 **	72.44 ***
Nutrient x Grass x RestMix$_{[2,279]}$	*37.47 ****	*3.05 **	19.71 ***	8.97 ***
Broadleaf x Grass x RestMix$_{[2,279]}$	*136.26 ****	*23.26 ****	27.86 ***	126.91 ***
Nutrient x Broadleaf x Time$_{[3,279]}$	*0.61*	*0.12*	0.56	0.12
Nutrient x Grass x Time$_{[3,279]}$	*0.58*	*0.08*	1.28	0.24
Broadleaf x Grass x Time$_{[3,279]}$	*0.17*	*0.06*	2.51	0.15
Nutrient x RestMix x Time$_{[6,279]}$	*0.21*	*0.02*	1.41	0.13
Broadleaf x RestMix x Time$_{[6,279]}$	*0.09*	*0.47*	1.02	0.16
Grass x RestMix x Time$_{[6,279]}$	*0.07*	*0.06*	1.09	0.13
Nutrient x Broadleaf x Grass x RestMix$_{[1,279]}$	*6.96 ***	*47.69 ****	46.327 ***	0.00
Nutrient x Broadleaf x Grass x Time$_{[3,279]}$	*0.62*	*0.43*	0.90	0.09
Nutrient x Broadleaf x RestMix x Time$_{[6,279]}$	*0.23*	*0.11*	0.59	0.08
Nutrient x Grass x RestMix x Time$_{[6,279]}$	*0.24*	*0.05*	0.76	0.28
Broadleaf x Grass x RestMix x Time$_{[6,279]}$	*0.16*	*0.18*	0.02	0.18
Nutrient x Broadlead x Grass x RestMix x Time$_{[3,279]}$	*0.22*	*0.43*	1.48	0.53

*Table-6.3.1. F-values and significance levels (0.000= ***, 0.001 = **, 0.05 = *, NS = not significant) for the main and interaction effects of time, weedy grass species, weedy broadleaf species, nutrient status and restoration seed mixture on the community-weighted means of seed mass, seedling relative growth rate, germination rate and 2C DNA.*

Model			Seed Mass (mg)			Seedling Relative Growth Rate (g/g/day)			Germination Rate (Number of Days)			2C DNA Content (pg)		
Weedy Broadleaf Species	Weedy Grass Species	Restoration Mix	$F_{[1,279]}$	PA	NE	$F_{[1,279]}$	PA	NE	$F_{[1,279]}$	PA	NE	$F_{[1,279]}$	PA	NE
Yes		*Loc*										204.12 ***	1.75± 0.16	4.40± 0.25
No	*No*								7.29 ***	2.02± 0.28	1.61± 0.16			
No		*ResB*				9.55 ***	1.23± 0.08	1.29± 0.04	127.17 ***	1.45± 0.09	1.04± 0.08			
	Yes	*ResA*				45.03 ***	0.61± 0.02	1.25± 0.02						
	Yes	*ResB*				35.91 ***	0.70± 0.03	1.35± 0.02				7.41 ***	1.83± 0.14	3.77± 0.43
	No	*ResA*				25.51 ***	1.15± 0.05	1.30± 0.03						
No	*No*	*Loc*	12.31 ***	2.05± 0.04	2.15± 0.03	23.69 ***	1.33± 0.01	1.29± 0.01				720.28 ***	7.72± 0.16	4.70± 0.14
No	*Yes*	*Loc*	220.79 ***	2.40± 0.09	1.59± 0.06	31.03 ***	1.37± 0.02	1.30± 0.01	77.01 ***	0.85± 0.12	3.15± 0.24	307.45 ***	4.20± 0.36	7.84± 0.24
No	*No*	*ResA*	67.41 ***	1.50± 0.11	2.98± 0.08							14.17 ***	2.11± 0.11	2.99± 0.28
No	*Yes*	*ResA*	661.95 ***	1.10± 0.22	1.45± 0.04				9.12 ***	0.96± 0.02	2.90± 0.12	2139.05 ***	0.41± 0.28	6.08± 0.12
No	*No*	*ResB*	608.57 ***	1.48± 0.05	2.08± 0.03							2574.01 ***	1.53± 0.07	3.57± 0.05
No	*Yes*	*ResB*	32.41 ***	2.28± 0.04	2.53± 0.03									
Yes	*No*	*Loc*	151.72 ***	1.18± 0.11	1.93± 0.04	133.59 ***	0.65± 0.02	1.123± 0.03	24.95 ***	3.31± 0.68	3.84± 0.28			
Yes	*Yes*	*Loc*	105.59 ***	1.19± 0.06	1.86± 0.06	202.19 ***	0.64± 0.02	1.08± 0.02						
Yes	*No*	*ResA*	334.30 ***	1.27± 0.43	1.77± 0.04				5.45 *	3.63± 0.38	4.38± 0.33	49.41 ***	3.12± 0.12	7.33± 0.33
Yes	*Yes*	*ResA*	89.27 ***	1.31± 0.40	2.03± 0.04				72.97 ***	0.72± 0.10	1.78± 0.09	24.80 ***	1.25± 0.43	1.72± 0.09
Yes	*No*	*ResB*	7.71 ***	0.71± 0.20	2.26± 0.06	6.13 *	0.44± 0.02	1.26± 0.02	101.47 ***	0.54± 0.01	4.58± 0.50	4.69 *	0.63± 0.09	4.89± 0.50
Yes	*Yes*	*ResB*	92.08 ***	0.11± 0.15	1.91± 0.06				17.94 ***	0.20± 0.02	3.23± 0.74			

*Table-6.3.2. F-values and significance levels (0.000= ***, 0.001 = **, 0.05 = *, NS = not significant) for the main effects of soil nutrient status on the community-weighted means of seed mass, seedling relative growth rate, germination rate and*

2C DNA content. Soil nutrient statuses averages ± standard errors are also reported.

6.3.1b. Weedy Broadleaf Species

The results of the main effects of weedy broadleaf species (Table-6.3.3.) demonstrate that there were significant impacts on the developing communities' seed and germination trait structure. Soil seed banks hosting weedy broadleaf species were found to produce communities similar to those inhabiting post-agricultural soils. The dominant species in the communities were faster germinators with small seeds and low nuclear DNA content. Given the lack of maternal resources, the relative growth rates were found to be slower than communities establishing on soils lacking weedy broadleaf species.

Model			Seed Mass (mg)			Seedling Relative Growth Rate (g/g/day)			Germination Rate (Number of Days)			2C DNA Content (pg)		
Nutrient Status	Weedy Grass Species	Restoration Mix	$F_{[1,279]}$	Yes	No	$F_{[1,279]}$	Yes	No	$F_{[1,279]}$	Yes	No	$F_{[1,279]}$	Yes	No
	Yes	*ResB*				12.71 ***	0.40± 0.03	1.33± 0.02						
	Yes	*ResA*				41.18 ***	1.03± 0.03	1.25± 0.02						
	No	*ResA*				31.20 ***	0.86± 0.02	1.30± 0.03						
NE	*Yes*					53.96 ***	1.17± 0.03	1.34± 0.02						
NE	*Yes*	*Loc*	250.86 ***	2.39± 0.09	1.59± 0.05				66.06 ***	1.05± 0.14	3.25± 0.01	230.61 ***	4.92± 0.38	8.20± 0.01
NE	*No*	*Loc*	160.38 ***	1.98± 0.04	1.92± 0.03				15.77***	3.85± 0.52	4.04± 0.01	235.39 ***	5.26± 0.38	4.86± 0.01
NE	*Yes*	*ResA*	202.10 ***	1.73± 0.06	1.24± 0.05				9.25 ***	1.99± 0.05	2.95± 0.03	1730.05 ***	1.40± 0.11	6.38± 0.03
NE	*No*	*ResA*	293.76 ***	1.22± 0.05	1.84± 0.04				20.44 ***	2.15± 0.05	1.68± 0.02	64.56 ***	9.73± 0.18	6.80± 0.18
NE	*Yes*	*ResB*	171.32 ***	0.74± 0.24	1.98± 0.05				30.27 ***	2.53± 0.37	3.04± 0.03			
NE	*No*	*ResB*	31.78 ***	2.08± 0.09	2.38± 0.05				163.81 ***	1.91± 0.09	1.26± 0.02	4.32 *	1.98± 0.76	4.14± 0.03
PA	*Yes*	*Loc*	93.85 ***	0.70± 0.07	1.87± 0.06							54.06 ***	3.59± 0.84	4.60± 0.02
PA	*No*	*Loc*	15.27 ***	1.16± 0.11	2.16± 0.04							241.78 ***	0.64± 0.05	2.72± 0.03
PA	*Yes*	*ResA*	13.82 ***	1.28± 0.39	2.24± 0.03				90.00 ***	0.79± 0.03	1.73± 0.01	245.16 ***	1.69± 0.15	5.54± 0.02
PA	*No*	*ResA*	41.42 ***	1.21± 0.44	1.91± 0.07				14.77 ***	3.31± 0.68	4.32± 0.05	41.45 ***	1.04± 0.22	1.42± 0.01
PA	*Yes*	*ResB*	6.39 ***	0.11± 0.39	2.37± 0.03									
PA	*No*	*ResB*	232.47 ***	1.27± 0.20	1.97± 0.04				86.60 ***	3.63± 0.38	4.38± 0.07	895.92 ***	1.69± 0.15	5.54± 0.02

*Table-6.3.3. F-values and significance levels (0.000= ***, 0.001 = **, 0.05 = *, NS = not significant) for the main effects of a weedy broadleaf seed bank on the community-weighted means of seed mass, seedling relative growth rate, germination rate and 2C DNA content. Presence/absence averages ± standard errors are also reported.*

6.3.1c. Weedy Grass Species

The results of the main effects of weedy broadleaf species (Table-6.3.4.) demonstrate that there were significant impacts on the developing communities'

seed and germination trait structure. The presence of weedy grass species in the soil seed bank was demonstrated to produce swards of quickly germinating small seeded species. The establishing seedling communities were revealed to have low relative growth rates and nuclear DNA content.

Model			Seed Mass (mg)			Seedling Relative Growth Rate (g/g/day)			Germination Rate (Number of Days)			2C DNA Content (pg)		
Nutrient Status	Weedy Broadleaf Species	Restoration Mix	$F_{[1,279]}$	Yes	No	$F_{[1,279]}$	Yes	No	$F_{[1,279]}$	Yes	No	$F_{[1,279]}$	Yes	No
	No	*ResB*							31.05 ***	1.05± 0.03	1.16± 0.07			
	Yes	*Loc*										84.08 ***	2.98± 0.22	4.40± 0.25
NE	*No*					14.89 ***	1.27± 0.01	1.29± 0.01						
NE	*Yes*					208.35 ***	0.95± 0.02	1.23± 0.03						
PA		*Loc*							7.49 ***	0.61± 0.05	1.46± 0.20			
NE	*No*	*Loc*	1591.48 ***	0.78± 0.02	1.52± 0.03				30.86 ***	5.45± 0.57	3.62± 0.13	2714.66 ***	11.49± 0.13	6.58± 0.13
NE	*Yes*	*Loc*	69.92 ***	2.39± 0.09	1.99± 0.06				33.44 ***	1.05± 0.14	3.67± 0.35			
NE	*No*	*ResA*	1037.87 ***	0.75± 0.03	1.61± 0.04				18.62 ***	3.90± 0.61	2.56± 0.33	224.00 ***	11.35± 0.21	7.61± 0.33
NE	*Yes*	*ResA*	26.06 ***	0.88± 0.23	1.47± 0.05							1629.54 ***	0.71± 0.32	5.57± 0.15
NE	*No*	*ResB*	22.28 ***	2.53± 0.03	2.61± 0.02							87.55 ***	4.34± 0.11	4.97± 0.08
NE	*Yes*	*ResB*	27.44 ***	0.74± 0.13	1.76± 0.09				18.22 ***	2.53± 0.37	3.48± 0.92			
PA	*No*	*Loc*	11.48 ***	1.25± 0.47	2.22± 0.07							82.34 ***	2.12± 0.22	5.96± 0.26
PA	*Yes*	*Loc*	333.56 ***	1.19± 0.06	1.81± 0.04	94.91 ***	0.64± 0.02	1.09± 0.04						
PA	*No*	*ResA*	26.68 ***	1.12± 0.21	1.83± 0.08	29.04 ***	0.59± 0.01	1.37± 0.05	20.60 ***	0.96± 0.02	1.97± 0.07	104.11 ***	0.40± 0.08	1.45± 0.07
PA	*No*	*ResB*	65.61 ***	1.34± 0.03	1.48±0 .05	10.02 ***	0.82± 0.04	1.11± 0.12				33.48 ***	1.25± 0.05	1.53± 0.07
PA	*Yes*	*ResB*							84.16 ***	0.20± 0.02	4.33± 0.33	35.61 ***	0.30± 0.04	4.33± 0.33
PA	*Yes*	*ResA*							17.95 ***	0.72± 0.10	4.08± 0.27	46.07 ***	1.25± 0.43	3.49± 0.27

*Table-6.3.4. F-values and significance levels (0.000= ***, 0.001 = **, 0.05 = *, NS = not significant) for the main effects of a weedy grass seed bank on the community-weighted means of seed mass, seedling relative growth rate, germination rate and 2C DNA content. Presence/absence averages ± standard errors are also reported.*

6.3.1c. Restoration Mix

The sowing of three different seed mixes was found to impact the seed and germination trait profile of establishing communities (Table-6.3.5.). ResB was reported to produce communities dominated by fast germinators and establishers; small seeded species with low nuclear DNA content, but quick germination rate and seedling relative growths. On the other hand, the Loc and ResA seed mixtures were found to support communities of the opposite seed and germination traits.

Model			Seed Mass (mg)				Seedling Relative Growth Rate (g/g/day)				Germination Rate (Number of Days)				2C DNA Content (pg)			
Nutrient Status	**Weedy Broadleaf Species**	**Weedy Grass Species**	**$F_{[2,279]}$**	**Loc**	**ResA**	**ResB**	**$F_{[2,279]}$**	**Loc**	**ResA**	**ResB**	**$F_{[2,279]}$**	**Loc**	**ResA**	**ResB**	**$F_{[2,279]}$**	**Loc**	**ResA**	**ResB**
	No	*No*									3.84 *	**A** 1.61± 0.16	**A** 1.34± 0.07	**B** 0.54± 0.05				
NE	*No*										38.99 ***	**A** 2.33± 0.23	**B** 1.28± 0.15	**C** 0.34± 0.03				
NE	*Yes*										29.05 ***	**A** 3.07± 0.32	**B** 2.19± 0.17	**C** 1.33± 0.16				
NE		*Yes*									14.20 ***	**A** 2.33± 0.19	**B** 1.47± 0.07	**C** 0.64± 0.04				
NE	*No*	*No*	30.95 ***	**A** 2.47± 0.03	**B** 2.58± 0.03	**C** 1.38± 0.06									23.73 ***	**A** 3.72± 0.21	**B** 4.74± 0.25	**C** 2.82± 0.04
PA	*No*	*No*	16.72 ***	**A** 1.68± 0.07	**A** 1.49± 0.08	**B** 0.80± 0.10	10.69 ***	**A** 1.35± 0.07	**A** 1.36± 0.10	**B** 0.58± 0.07					685.34 ***	**A** 3.79± 0.11	**B** 1.82± 0.09	**C** 0.78± 0.05
NE	*Yes*	*No*	32.83 ***	**A** 1.63± 0.06	**A** 1.65± 0.07	**B** 1.16± 0.43					14.20 ***	**A** 3.45± 0.34	**B** 2.03± 0.07	**C** 0.97± 0.06	26.16 ***	**A** 7.12± 0.44	**A** 6.66± 0.51	**B** 1.81± 0.61
PA	*Yes*	*No*	3.65 *	**A** 2.35± 0.04	**A** 2.39± 0.03	**B** 1.44± 0.15	3.81 *	**A** 1.31± 0.02	**A** 1.28± 0.01	**B** 0.70± 0.03	11.45 ***	**A** 5.08± 0.71	**A** 6.93± 1.04	**B** 3.65± 0.40	12.47 ***	**A** 4.83± 0.29	**B** 5.56± 0.32	**C** 3.11± 0.42
NE	*No*	*Yes*	1112.37 ***	**A** 1.35± 0.03	**A** 1.11± 0.15	**B** 0.92± 0.19					18.12 ***	**A** 3.46± 0.41	**B** 1.65± 0.22	**C** 0.37± 0.04	569.81 ***	**A** 9.06± 0.15	**B** 5.24± 0.28	**C** 1.46± 0.34
NE	*Yes*	*Yes*	38.73 ***	**A** 1.85± 0.08	**B** 1.07± 0.11	**C** 0.52± 0.10					28.09 ***	**A** 2.70± 0.30	**A** 2.35± 0.26	**B** 1.70± 0.26	10.95 ***	**A** 3.42± 0.49	**B** 1.79± 0.38	**B** 1.33± 0.35
PA	*Yes*	*Yes*	437.77 ***	**A** 2.01± 0.03	**B** 1.21± 0.43	**B** 0.24± 0.30	90.24 ***	**A** 1.10± 0.02	**B** 0.64± 0.02	**B** 0.40± 0.01	11.79 ***	**A** 1.35± 0.07	**B** 0.79± 0.02	**C** 0.10± 0.01	7.11 ***	**A** 1.92± 0.20	**B** 1.04± 0.08	**C** 0.10± 0.08
PA	*No*	*Yes*					40.25 ***	**A** 1.25± 0.02	**A** 1.18± 0.02	**B** 0.64± 0.02	39.27 ***	**A** 1.37± 0.07	**A** 1.66± 0.10	**B** 0.72± 0.10	46.53 ***	**A** 2.50± 0.19	**B** 1.63± 0.26	**C** 1.25± 0.29

*Table-6.4.5. F-values and significance levels (0.000= ***, 0.001 = **, 0.05 = *, NS = not significant) for the main effects of restoration mixes on the community-weighted means of seed mass, seedling relative growth rate, germination rate and 2C DNA content. Restoration mix averages ± standard errors are also reported. Bolded letters note the Tukey HSD groupings.*

6.3.1d. Summary of Main Effects on Seed and Germination Traits

A summary of the results of the analysis of variance investigating the main effects of soil nutrient status, composition of the seed bank and the restoration mixes on the seed and germination traits is displayed in Figure-6.3.1. Collectively, the findings suggest that post agricultural soils with a seed bank hosting weedy broadleaf and grass species will generally produce small seeded communities of low nuclear DNA content and slow relative growth rates. This is further exacerbated, however, with sowing the ResB seed mixture. Cross-examining the results in Figure-6.4.1 highlights a significant difference in the impacts of weedy broadleaf and grass species on the germination rate of dominant species. The presence of weedy grass species was found to increase germination rate of the community, whereas broadleaf species increased the number of days till half the population has germinated.

These general findings are consistent over a large proportion of the results, but there are some discrepancies that postulate that the combination of NE soil nutrient status and Loc or ResA seed mix can mitigate the effects of weedy species on the seed and germination traits of assemblages. For example, Table-6.3.3. and 6.3.4. highlight that NE and Loc have the capacity to increase community seed mass, seedling relative growth rate, nuclear DNA content and reducing germination rate.

	Nutrient Status		**Weedy Broadleaf Species**		**Weedy Grass Species**		**Restoration Mix**		
	PA	*NE*	*Yes*	*No*	*Yes*	*No*	*Loc*	*ResA*	*ResB*
Seed Mass (mg)	↓	↑	↓	↑	↓	↑	↑	↑	↓
Seedling Relative Growth Rate (g/g/day)	↓	↑	↓	↑	↓	↑	↓	↓	↑
Germination Rate (Number of Days)	↑	↓	↑	↓	↓	↑	↓	↓	↑
2C DNA Content (pg)	↓	↑	↓	↑	↓	↑	↑	↑	↓

Figure-6.3.1. Summary of the main effects of nutrient status, soil seed bank composition (weedy broadleaf or grass species) and restoration mix on the community-weighted means of seed mass, seedling relative growth rate, germination rate and 2C DNA content.

6.4.2. Modelling the Early Stages of Grassland Restoration

Table-6.4.5. shows the best MaxEnt models, and their plant trait composition, which predicted the relative abundances of species. The Pearson R^2 highlights the amount of variance that was successfully explained between observed and predicted relative abundances in the data observed from the microcosms. Significant results were found for numbers of plant traits and explained between 31-98% of variation and therefore rejects the null hypothesis of the MaxEnt model that community assembly processes are random.

The appropriate number of plant traits was investigated using an analysis of variance followed by a Tukey HSD test. The findings revealed that the number of traits significantly affects the total explained variation and the Tukey HSD demonstrated that seven is an optimal number in MaxEnt modelling approaches, however, three traits encapsulating both mature and seed traits will also suffice (+80%). Table-6.3.6. indicates that seed mass did not heavily feature in the models.

Number of Traits	Plant Traits	R^2
One	*Leaf Dry Matt Content*	0.31 *
Two	*Leaf Dry Matter Content, Germination Rate*	0.66 **
Three	*Leaf Dry Matter Content, Germination Rate, Plant Height*	0.82 ***
Four	*Leaf Dry Matter Content, Leaf Nitrogen Content, Leaf C:N, 2C DNA Content*	0.89 ***
Five	*Plant Height, Leaf Dry Matter Content, Leaf Nitrogen Content, Leaf C:N, Seedling Relative Growth Rate*	0.92 ***
Six	*Leaf Dry Matter Content, Specific Leaf Areas, Leaf Thickness, Seedling Relative Growth Rate, Germination Rate*	0.96 ***
Seven	*Leaf Dry Matter Content, Specific Leaf Area, Leaf Thickness, Leaf Nitrogen Content, Leaf C:N , Seedling Relative Growth Rate, Germination Rate*	0.97 ***
Eight	*Leaf Dry Matter Content, Specific Leaf Area, Leaf Thickness, Leaf Nitrogen Content , Leaf C:N, Seedling, Relative Growth Rate , 2C DNA Content, Germination Rate*	0.98 ***
Nine	*Plant Height , Leaf Dry Matter Content , Specific Leaf Area, Leaf Thickness, Leaf Nitrogen Content, Leaf C:N, Seedling Relative Growth Rate, 2C DNA Content , Germination Rate*	0.98 ***
Ten	*Full*	0.98 ***

*Table-6.3.6. Best models for each number of plant traits found from the maximum entropy modelling approach. R^2 values are reported together with significance levels (0.000= ***, 0.001 = **, 0.05 = *, NS = not significant) for comparison. Seven traits were found by Tukey HSD and is highlighted as the optimal number of traits.*

6.3.3. Examining the Progression Towards Restoration Targets

The four-way analysis of variance found significant interactions at the three (functional evenness), two (Bray-Curtis) and single factor (functional richness and divergence) levels. The interaction effects were decomposed to analyse the main effects of nutrient status, weedy broadleaf species, weedy grass species and restoration time. These results are detailed and interpreted in sections 6.3.3a to 3c.

Model$_{[df]}$	**Bray-Curtis**	**Functional Richness**	**Functional Evenness**	**Functional Divergence**
Time$_{[3,344]}$	*40.83 ****	*1.01*	57.56 ***	57.975 ***
Weedy Grass Species$_{[1,344]}$	*570.71 ****	*2.40*	2.53	1.35
Weedy Broadleaf Species$_{[1,344]}$	*713.79 ****	*0.01*	0.31	2.43
Nutrient Status$_{[1,344]}$	*5.65 **	*8.44 **	3.83	1.56
Time x Grass$_{[3,344]}$	*3.70 **	*0.40*	0.03	0.82
Time x Broadleaf$_{[3,344]}$	*4.82 ***	*0.02*	0.36	0.20
Grass x Broadleaf$_{[1,344]}$	*336.78 ****	*3.30*	4.56 *	1.74
Time x Nutrient$_{[3,344]}$	*0.134*	*0.05*	0.75	1.33
Grass x Nutrient$_{1,344]}$	*15.22 ****	*0.23*	2.30	1.19
Broadleaf x Nutrient$_{[1,344]}$	*32.11 ****	*1.29*	0.24	0.36
Time x Grass x Broadleaf$_{[3,344]}$	*1.75*	*0.03*	3.42 *	2.01
Time x Grass x Nutrient$_{[3,344]}$	*0.47*	*0.09*	0.76	0.35
Time x Broadleaf Nutrient$_{[3,344]}$	*1.72*	*0.07*	0.64	1.11
Grass x Broadleaf x Nutrient$_{[1,344]}$	*0.18*	*1.94*	1.32	0.03
Time x Grass x Broadleaf x Nutrient$_{[3,344]}$	*1.60*	*0.13*	0.18	0.06

*Table-6.3.7. F-values and significance levels (0.000= ***, 0.001 = **, 0.05 = *, NS = not significant) for the main and interaction effects of time, weedy grass species, weedy broadleaf species and nutrient status on the progression towards vegetation (Bray-Curtis) and functional (functional richness, evenness and divergence) restoration targets.*

6.3.3a. Seedbank Composition

The results (Table-6.3.8) demonstrated that weedy broadleaf and grass species have significant detrimental effects on the progression towards vegetation structure and functional restoration targets. These impacts are particularly evident in the composition of the seed bank. The absence of weedy species in the seed bank had a significant and positive effect on the progression towards the vegetation targets; approximately a 25% improvement in both cases. The absence of weedy species was also found to positively influence progression towards functional targets (functional evenness) across a limited number of scenarios. This was true for the lack of weedy broadleaf species and significant differences were detected after eight weeks in the absence of weedy grass species.

Model					**Bray-Curtis**			**Functional Evenness**		
Nutrient Status	**Time (Weeks)**	**Weedy Broadleaf Species**	**Weedy Grass Species**	**Response**	**$F_{[1,344]}$**	**Yes**	**No**	**$F_{[1,344]}$**	**Yes**	**No**
				Broadleaf	54.25 ***	0.95± 0.01	0.69± 0.04			
	8		*No*	*Broadleaf*				4.46 *	0.21± 0.03	0.12± 0.06
				Grass	101.14 ***	0.97± 0.02	0.72± 0.03			
		No		*Grass*				6.54 *	0.32± 0.06	0.25± 0.04

*Table-6.3.8. F-values and significance levels (0.000= ***, 0.001 = **, 0.05 = *, NS = not significant) for the main effects of weedy grass and broadleaf seed banks on the progression towards vegetation (Bray-Curtis) and functional (functional evenness) restoration targets. Presence/absence averages ± standard errors are also reported.*

6.3.3b. Nutrient Status

The findings for the effect of nutrient status demonstrate that the influence of nutrient status is dependent on the structure and composition of the soil seed bank (Table-6.4.9). The presence of weedy grass species was found to demonstrate a positive influence of the post agricultural nutrient status towards the target floristic structure and composition. However, the presence of weedy broadleaf species reverted this relationship. The positive influence of the NE condition, however, was reported for a functional target (FRic) (Figure-6.3.2.).

Model		**Bray-Curtis**		
Weedy Broadleaf Species	**Weedy Grass Species**	**$F_{[1,344]}$**	**PA**	**NE**
	Yes	10.59 ***	0.89± 0.03	0.94± 0.01
No		8.92 ***	0.65± 0.03	0.72± 0.04
Yes		15.00 ***	0.97± 0.01	0.93± 0.01

*Table-6.3.9. F-values and significance levels (0.000= ***, 0.001 = **, 0.05 = *, NS = not significant) for the main effects of soil nutrient status on the progression towards vegetation (Bray-Curtis) restoration target. Nutrient status' averages ± standard errors are also reported.*

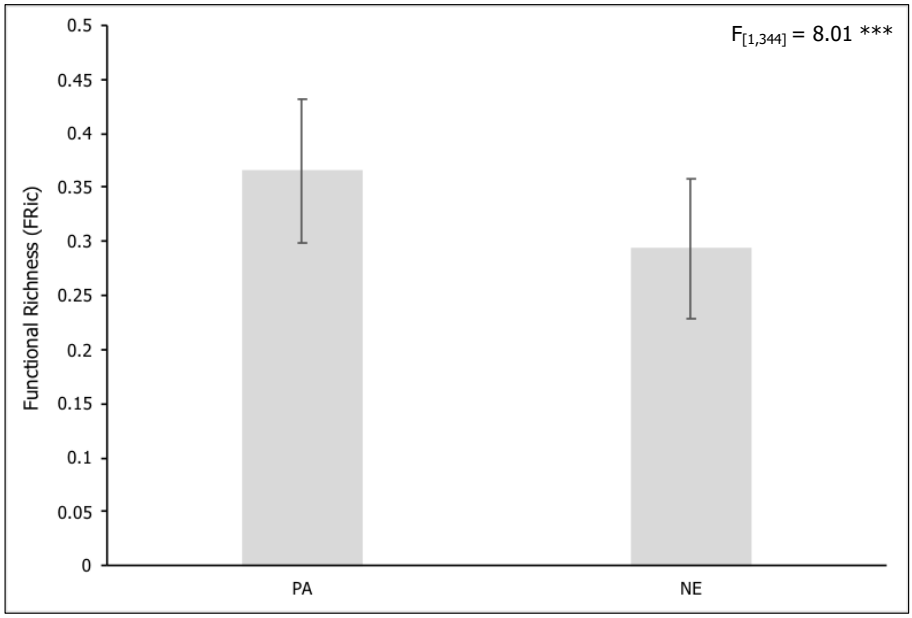

Figure-6.3.2. Main effects of soil nutrient status on the progression towards functional (functional richness) restoration target. Errors bars illustrate standard errors.

6.3.3c. Time

The main effects of the analysis of variance revealed that the first month can show considerable progression towards functional targets. Functional divergence (Figure-6.3.3.) found a large and significant difference between two and four weeks as revealed by Tukey's HSD. This was also reported for functional evenness (Table-6.3.10.) under the control (no weedy species) and saturated (both weedy broadleaf and grass species) seed banks. For these conditions, the first four weeks recorded circa 0.15 per week progression towards the functional target that was five times quicker than the presence of weedy broadleaf and grass species individually (0.03 per week). Additionally, Tukey's HSD indicated significant differences were exposed after six weeks.

Model		Functional Evenness				
Weedy Broadleaf Species	**Weedy Grass Species**	**$F_{[3,344]}$**	**2**	**4**	**6**	**8**
No	*No*	32.69 ***	0.50± 0.05	0.21± 0.04	0.18± 0.04	0.12± 0.03
Yes	*No*	6.55 ***	0.45± 0.06	0.31± 0.05	0.23± 0.06	0.21± 0.06
No	*Yes*	7.20 ***	0.46± 0.06	0.34± 0.07	0.26± 0.05	0.21± 0.05
Yes	*Yes*	26.38 ***	0.53± 0.04	0.24± 0.06	0.20± 0.04	0.19± 0.04

*Table-6.3.10. F-values and significance levels (0.000= ***, 0.001 = **, 0.05 = *, NS = not significant) for the main effects of time (number of weeks) on the progression towards functional (functional evenness) restoration targets. Biweekly averages ± standard errors are reported.*

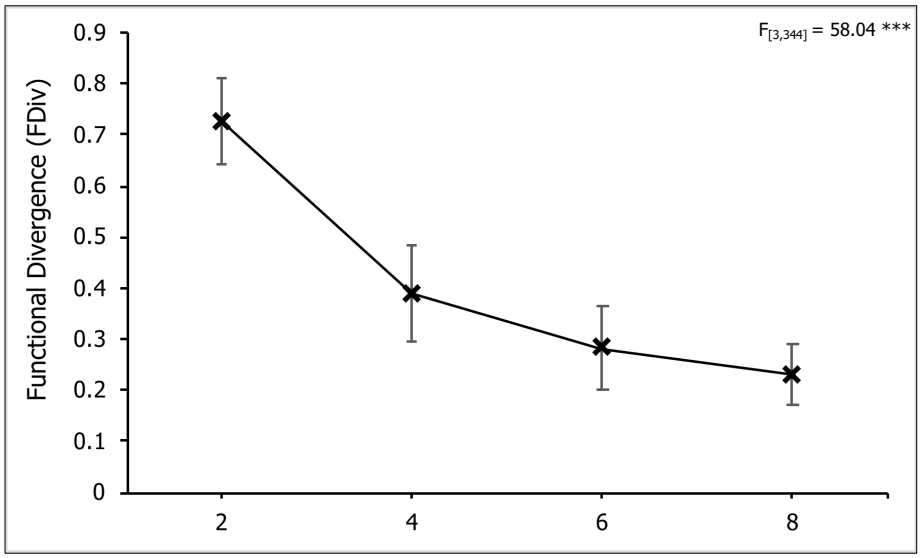

Figure-6.3.3. Main effects of time (number of weeks) on the progression towards functional (functional divergence) restoration target. Errors bars illustrate standard errors.

6.5. Discussion

Seed mass is the most commonly implemented plant trait in germination and establishment studies and is thought of as a strong predictor of germination and establishment processes (Jiménez-Alfaro *et al.*, 2016). Saatkamp *et al* (2011) urged greater attention to be paid to specific germination traits, and their underrepresentation is evident in the TRY database. Numerous authors have now indicated the importance of germination and seedling plant traits in understanding the impacts of environmental parameters in the early phases of grassland restoration projects and predicting species' relative abundances (Olff *et al.*, 1994; Sonnier, Shipley and Navas, 2010). This chapter aimed to reveal the trait-based assembly of temperate grassland communities and understand the impacts of abiotic and biotic factors during seed germination and establishment, and their effects on success in the early stages of temperate grassland restoration experiment. Three hypotheses were proposed and each of these is discussed with reference to the main findings in the coming sections.

6.5.1. Non-Random Assembly of Temperate Grasslands

Community assembly is considered to be non-random and this was conceptualised in the trait-based Response-Effect Framework (Lavorel and Garnier, 2002). Species are subjected to a series of barriers that underpin assembly rules (dispersal, habitat and biotic filters) and the community-level trait values are a product of sorting. The maximum entropy model mathematically defined trait-based community assembly through the prediction of relative species abundances according to species mean trait values (Shipley, Vile and Garnier, 2006). Sonnier, Shipley and Navas (2010) highlighted a lack of definition in the number and identity of plant traits needed to accurately predict species' relative abundance. In light of this, this chapter investigated this claim using seed, germination and mature plant traits. It was hypothesised that species relative abundances will be most accurately predicted by a model inclusive of these three trait classes. The findings of the MaxEnt modelling support the original hypothesis and indicate the detection of non-random trait-based assembly processes. The optimal MaxEnt model was found to include seven traits. This coincides with Laughlin *et al*'s (2015) postulation that between five to eight plant traits was an optimal level. Interspecific trait differences have previously

been reported to reveal deterministic assembly process, particularly with traits governing a species' immigration, survival and growth potential (Sonnier, Shipley and Navas, 2010). The result of the optimal MaxEnt model echoed these finding and suggests leaf traits together with germination rate and seedling relative growth rate can accurately predict species' relative abundances (97%). It is clear, therefore, that the first mathematical model of trait-based community assembly is accurate, however since its inception the MaxEnt model has been scrutinised and thus more advanced modelling approaches have been devised.

The MaxEnt model of community assembly has been criticised on two fronts. It largely ignores intraspecific trait variation, which is continually highlighted as an important source of trait variation, and is dependent on the measurement of community structure and calculation of community-weighted means in order to predict community assembly in other sites (Albert *et al.*, 2010; Messier, McGill and Lechowicz, 2010; Laughlin *et al.*, 2012). Laughlin *et al* (2012) proposed a flexible solution to the MaxEnt model for predicting relative abundances of species in a regional pool. The Traitspace model provided a general framework unifying coexistence theory, evolutionary biology and trait-based approaches. The model was based on a hierarchical Bayesian approach that combined intraspecific trait distributions and the relationship between individual level traits and the environment. The Traitspace was found to demonstrate a clear selection of traits along the gradient of mean annual temperature and allowed the quantification of the strength of environmental filters, and the joint effects of multiple gradients (Laughlin *et al.*, 2012). It has been found that niche differentiation (biotic filtering) can be revealed through the analysis of bimodal distributions of intraspecific plant traits for a range of environmental conditions (Laughlin *et al.*, 2015).

6.5.2. Abiotic and Biotic Impacts on Seed and Germination Trait Profiles

Seed traits have been prominent figures in functional ecology to quantify a species germination, establishment, regeneration and colonisation abilities (Pywell *et al.*, 2003; Storkey, 2004; Cole, Lunt and Koen, 2005). In recent decades, seed mass has been thought to sufficiently capture these capabilities, but Jiménez-Alfaro *et al* (2016) recommended expanding this suite of plant traits to directly measure

germination characteristics. This chapter concurred with Jiménez-Alfaro *et al* (2016) and created a unique trait profile to investigate the abiotic and biotic effects on the seed and germination traits of dominant species. It was hypothesised that the edaphic conditions typical of post-agriculture (high soil nutrient status and weedy seed banks) and sowing of a non-local seed mixture (ResA and ResB) will produce communities dominated by those able to efficiently exploit fleeting resources and quickly establish populations. The findings of this chapter partially satisfy this original hypothesis and suggested that communities quickly establishing on post-agriculture soil and/or in the response to sowing of ResB resemble those of weak competitors and opportunistic weedy plant species. This is exemplified in the increased abundance of small seeded and slow growing seedlings. Erikson and Eriksson (1997) found that this trait profile typically manifested in plant species adapted for long periods of dormancy and thus persistency. A widespread soil seed bank survey reiterated Erikson and Erikson's findings reporting that post-agriculture soils were saturated with smaller-seeded plant species and emerged in occasions where the dispersal and habitat filters were compromised, such as following a community disturbance event like grazing, firing or mowing (Rees, 1996). One could argue that the artificial setting of this experiment bypassed the dispersal and habitat filters of temperate grasslands; an ample supply of nutrients with an abundance of open niche spaces created a window for weak competitors and/or opportunistic species to establish. Such has been reported following sward disturbance of temperate grasslands that found an increase of arable weeds and ruderality in the short term (Klaus *et al.*, 2017).

Scholars have suggested these ruderal species are vital to kickstart succession in high nutrient environments (Kimball *et al.*, 2014). The pioneering vegetation reduces the plant available nitrogen and causes a floristic shift favouring mid- and/or late-successional species (Rowe, Brown and Paschke, 2009). The competitive processes between early and mid/late-successional species is considered to play an important role in community dynamics and the abundance of arable weeds in temperate grassland communities indicates an early seral stage (Kleijin, 2003). Arable weeds are suggested to decline rapidly over the first two growing seasons but are able to persist as residual populations via sporadic germination from a saturated seed bank (Klaus *et al.*, 2017). This trade-off between

lifespan and seed bank longevity has been frequently reported for European temperate grasslands (Thompson, Bakker and Hodgson, 1998). Issues remain, however, in the measures and recordings of seed bank longevity – suggested only 46% is known for north western Europe floras (Thompson, Bakker and Bekker, 1997). Temperate grassland seed banks are not typically viewed as persistent as shown in dry and acid target grasslands by Valkó *et al* (2010). Furthermore the proportions of individual species and their viability in the seed bank are greatly unknown and unexplored (Pyke, Brooks and D'Antonio, 2010). Despite this, a seed bank dominated by ruderal species are thought to not inhibit the recovery of grassland species due to their short lifespan. Cherwin *et al* (2009) found that arable weeds and seed bank survivors only occupied 8% of vegetation cover following five years of grassland restoration efforts in Colorado (United States). Furthermore, it is postulated that the seed bank lags considerably behind vegetation development as reported by Wagner, Walker and Pywell (2017) who found limited convergence between the soil seed bank and vegetation over seven years of study. On the contrary, Kleijin (2003) found that in the long-term post-agricultural environments stagnated at a species-poor and ruderal stage.

Disparities in the published literature is common in reporting the short- and long-term outcomes of grassland restoration projects, and this is exacerbated by a lack of publication in failed projects (Maron *et al.*, 2012). Given the short nature of this chapter's experiments, the wider implications and future scenarios can only be inferred from an inconsistent research bank. Future developments should extend the number of growing seasons beyond the first year adequately investigate the impacts of agricultural soil legacies in the long-term, speculated to be in the region of five to ten years (Rowe and Holland, 2013). Despite this, the findings of this chapter can still question whether reversing agricultural legacies and sowing a local seed mixture can produce communities dominated by late successional species.

Communities establishing on restored soil conditions and/or in response to the sowing of a local seed mixture were found to be dominated by large seeded species with high DNA content and quick metabolism (fast relative growth rate and germination rate). This chapter postulates these communities resemble those of

later successional stages. Lowering soil nitrogen content has been found to accelerate the rate succession in disturbed areas favouring late successional species that are often found on lower nutrient soils (Herron *et al.*, 2001). The combination of reduced soil fertility and the trait profile revealed in this chapter is thought to grant later successional species with the competitive edge; larger seed mass is positively linked with seedling biomass and establishment vigour (Westoby *et al.*, 1996). Greater maternal resources have been linked to increased plasticity in the establishment phase as the individual can preserve surplus resources to mitigate unfavourable conditions, such as carbon deficit, and strengthen the investment into capturing a limiting resource (Jurado and Westoby, 1992; Pywell *et al.*, 2003). This chapter postulates physical space may have been a limiting resource, given the small area of the microcosms, and aligned with previous scholars on this matter (Schippers, Snoeijing and Kropff, 1999). Higher seedling relative growth rates enable quicker exploitation of open niches, both above and below ground. Space was found to be a key factor underpinning competitive outcomes from cellular automaton models and suggested that monitoring of species' spatial distributions during competition experiments is needed (Silvertown *et al.*, 1992). This would be an appropriate development in the methodology of the current chapter as the microcosms were small in size (0.225 X 0.08m).

6.5.3. Progression Towards Restoration Targets

Vegetation structure and composition has taken precedence in determining the success of a restoration project (Prober and Thiele, 2005). Such is defined by species richness and composition and scholars have urged the movement away from taxon-specific targets (Block *et al.*, 2001; Babin-Fenske and Anand, 2010; Yurkonis, Wilsey, Moloney, Drobney, *et al.*, 2010). Functional targets have been at the forefront of recent efforts defining ideal communities based on the pristine and natural states of assemblages dictated by history (Jackson and Hobbs, 2009). This chapter aimed to investigate the main effects of soil nutrient status, weed seedbank composition and restoration age on restoration success as defined by the taxonomic and functional structure of restoration seed mixtures. Subsequently, it was hypothesised that a strong agricultural soil legacy would greatly hinder the progression towards both vegetation and functional targets. The results of this chapter generally support this hypothesis and highlights certain conditions that are

unfavourable for restoration. Progression towards vegetation targets was found to be adversely affected by a weedy seed bank (both grass or broadleaf species) especially when weedy broadleaf species are present in a high nutrient status soil. Similarly, functional targets' progression was found to be greater in restored edaphic environments (reduced soil nutrient status and a vacant soil seed bank) after eight weeks. In essence, these results advocate for active measures in the ecological restoration of the edaphic environment, with particular focus on eliminating weedy species from the soil seed bank. Additionally, this chapter provides empirical support for the efforts spent on removing undesirable plant species in ecological restoration projects – suggested to be 25% (Price and Weltzin, 2003).

Methods to eradicate a weedy seed bank has focused on topsoil removal, inversion and solarisation (Lambrecht and D'Amore, 2010; Olsson and Ödman, 2014). Solar heat sterilisation is the process of heating the top layer of soil to a point whereby seed survivability is negligible (Marushia and Allen, 2011). Lambrecht and D'Amore (2010) found that solarisation was effective in managing *Lolium perenne* populations and prevented long-term establishment from the seed bank. Such approaches have been criticised given the short lifespan and persistency of arable weeds in temperate grassland communities, instead practitioners are preoccupied on restoring the edaphic conditions likely to have an impact on short term restoration success, such as reducing soil nutrient status.

Reducing soil fertility status is a common feature in grassland restoration projects. Soil nitrogen availability is thought to be the most critical environmental filter as it greatly limits the establishment of mid to late successional species that are typically targeted (Rowe, Brown and Paschke, 2009; Cleland, Larios and Suding, 2013). Soil nitrogen is traditionally reduced through the introduction of a carbon (sucrose, straw, sawdust and mulch) to promote microbial immobilisation of soil nitrogen, which was found to hinder nitrophillic plant species that are often weedy (Bleier and Jackson, 2007; Doll *et al.*, 2011). Cover covers, such as soybean-corn rotations, have also been employed to ameliorate the soil nutrient status (Morris and Schupp, 2009). However, restoration ecologists and managers have reported a lack of

success in the short term with nitrogen immobilisation by mulching (Cione, Padgett and Allen, 2002). Averett *et al* (2004) did find that sawdust addition caused a 94% reduction in annual net nitrogen mineralisation that decreased undesirable species abundances by 40% in the second growing season. These findings postulate that success or failure of a restoration project cannot be accurately determined in the short term (Déri *et al.*, 2011).

Nemec and Bragg (2008) suggested that current ecological indicators of success (vegetation, species richness and diversity, etc.) only become viable after five to ten years (Block *et al.*, 2001; Lengyel *et al.*, 2012). Most restoration projects are tasked with restoring a historical state within five years due to the lack of long-term funding (Conrad and Tischew, 2011). This has produced research with suboptimal designs, statistical rigour, and a lack of long-term monitoring. Hilderbrand *et al* (2005) claimed the short-term view of restoration managers and stakeholders is rooted in the Clementsian model of succession, which defines ecosystems' development in a static and predictable fashion towards an end point or climax community. To ecologists, this concept is outdated but is suggested to have found new advocates among the authors of restoration plans (Choi, 2004; Hilderbrand, Watts and Randle, 2005). The assumption that ecosystems develop predictably has been reported in prairie restorations with regards to community composition and structure, but not for ecosystem processes and services (Derner *et al.*, 2004). Sayre (2010) since has scrutinised ecological restoration for producing aesthetically compelling but ecologically mislead results.

Sluis (2002) argued that adopting a functional perspective introduces an element of objectivity into restoration projects. Reformulating taxon-specific targets into functional ones instils a more dynamic and tangible view of ecosystems that centres on the production of specific or numerous ecosystem processes and services. Additionally, functional diversity measures have been suggested to be more accurate indicators of community stability, invasibility and functioning (Young *et al.*, 2009). Restoration projects adopting a functional approach, however, are severely lacking and understanding what environmental factors constrain restoration success is poor. The results of this chapter found that employing the same techniques to

restore vegetation structure will assist the progression towards functional targets. These findings, however, should be interpreted with caution as the functional targets were calculated from floristic data and failed to measure an ecosystem process or service. It is recommended future studies explicitly assess the provisioning of an ecosystem service, such as biomass production, and compare outputs with natural and/or target temperate grasslands. One suspects the findings of such investigation will provide valuable insights into the ecological restoration of temperate grasslands. Informed decisions can then be made on whether the rehabilitation of agricultural soil legacies is an appropriate action with respect to mimicking the biomass production of target grasslands.

6.4.5. Future Directions

6.4.5a. Accounting for the Soil Microbiota

Intensive management of temperate grasslands has greatly modified the composition of the microbial food web. Tilling has devastated arbuscular mycorrhizal fungi populations, reduced the abundance of collembolan and increased the number of parasitic nematodes (Barrett *et al.*, 2009; Middleton, Bever and Schultz, 2010; Paluch, Thomsen and Volk, 2013). This changed community is surmised to limit the germination and establishment of grassland plant species by impacting seed survival and selectively promoting or suppressing the growth of plant species (Murray, Thrall and Woods, 2001; Kardol, Bezemer and Van Der Putten, 2009).

Arbuscular mycorrhizal fungi are the most extensively researched microbial group in the edaphic environment. They form symbiotic relationships with specific plant species that can grant them a competitive advantage. The fungal hyphae penetrates or forms a sheath around the plants roots that ramifies into the mineral matrix – stabilising soil structure (Harris, 2009). In this relationship, the fungus gains photosynthate with the exchange of mineral nutrients, water and protection from root pathogens (Harris, 2009; Paluch, Thomsen and Volk, 2013). Arbuscular mycorrhizal fungi -plant relations are unique with differential plant responses to infection; late successional grassland species are suggested to be more responsive

and had increased competition over early successional plant species (Rowe, Brown and Claassen, 2007; Desserud and Naeth, 2012). The added establishment advantage is thought to stem from enhanced mineral uptake with AMF infection, as seen in transplanting inoculated *Sporobolus wrightii* and the associated absorption of phosphorus, nitrogen and zinc (Richter and Stutz, 2002). This inevitably sculpts the diversity and composition of the grassland sward (O'dea, 2007).

Restoration ecology has recognised the utility of the soil microbiota and reversing the microbiological legacy of intensive management has thus become a focus in recent years (Kulmatiski, 2011). Soil inocula from local temperate grassland communities or commercial suppliers have been common techniques. However, unsuccessful colonisation of inocula has been reported from commercial stocks, whereas locally collected inocula were found to infect and increase the coverage of native perennial plant species (Irvine *et al.*, 2013; Paluch, Thomsen and Volk, 2013). Traditionally a few individuals (cover crop, nurse species or nursery grown plugs) are inoculated and assumed to outsource to the neighbouring plants and the landscape – nucleation model (Middleton, Bever and Schultz, 2010). However, very little is known about the ability of AMF to colonise and persist in restored intensively managed landscapes; research from restored (inoculated) agricultural land still shows low fungal diversity six years after restoration efforts began (Barrett *et al.*, 2009).

General Discussion

Functional ecology has been praised as the 'Holy Grail' as it unites theories of community ecology and ecosystem science (Lavorel and Garnier, 2002). The popularisation of the sub-discipline has stimulated the publication and standardised manuals on measuring plant traits (Pérez-Harguindeguy *et al.*, 2013; Garnier *et al.*, 2017). Subsequently, this has led to the compilation of local and regional plant trait data - the TRY initiative is currently the largest collection of trait recordings; over five for 1100 traits and 2.6 million individuals spanning 100,000 plant species (Kattge *et al.*, 2011).

The Response-Effect Framework, proposed by Lavorel & Garnier (2002), demonstrated how trait-based filtering of 'response traits' induces patterns of convergence and divergence that shape plant communities' functional structure and composition. The incorporation of 'effect traits' elicited the impacts plant traits can have individually, and collectively on ecosystem processes and services. Functional ecology is still a relatively young discipline and scholars are continually urging research to adopt a trait-based approach.

This thesis aimed to investigate whether a trait-based approach can reveal the determining factors of community composition and ecosystem services provision in temperate grasslands. Using an array of datasets (National Vegetation Classification, the Park Grass Experiment, and the North Wyke Farming Platform), a plethora of environmental and management variables were analysed for their impacts on the expression of plant traits at the community-level, and on their subsequent translation into single- and multi-trait indices. Collectively, these were used to investigate the relationship between environmental factors, plant traits, biomass and livestock production. The results of these experiments have been discussed in great detail in their associated chapters but an overview of the major findings and issues regarding the overlap in response and effect traits, intraspecific variation and phylogenetic corrections.

7.1. Main Findings

7.1.1. Chapter Three

Ecological strategies describe a species or individual's fitness as dictated by their potential to grow, survive and reproduce (Westoby, 1998). These strategies have found considerable support globally, especially the Leaf Economics Spectrum, however, questions remain about the applicability of these strategies at all spatial and organisational levels (Díaz *et al.*, 2004, 2015; Wright *et al.*, 2004; Freschet *et al.*, 2010). Using temperate grasslands as an example, this chapter found that the seven plant traits studied were independent and so challenges the simplicity generalisability of current ecological strategies and published research. These plant traits were used to quantify and assess redundancy in univariate and multivariate functional diversity indices (Mouillot *et al.*, 2005; Villéger, Mason and Mouillot, 2008). It was found that multivariate functional divergence measures were strongly correlated and thus this these adapted accordingly to avoid multicollinearity in subsequent regression analyses.

7.1.2. Chapter Four

The Response-Effect Framework postulated the functional structure and composition of temperate grasslands are governed by a hierarchy of filters (Lavorel and Garnier, 2002). Ecological strategies have defined the changes in response traits for a limited number of abiotic filters and evidence for the biotic filter has been patchy (Louault *et al.*, 2005; Valladares and Niinemets, 2008; Maire *et al.*, 2009; Gommers *et al.*, 2013). This chapter tested the convergence-divergence paradox integral to the Response-Effect Framework in response to grazing and different fertilisation conditions. It was found plant trait patterning the conventional tolerance/avoidance strategy to grazing at either extreme, but the aftermath grazing condition was an amalgamation of the two trait profiles. This suggested the need to study plant trait responses along a grazing continuum varying in frequency and intensity. Improvement of temperate grasslands, particularly with mineral fertilisers, was found to stimulate an exploitative response in plant trait values. Conversely, the use of farmyard manure and ammonium sulphate-based fertiliser encourages the conservative strategy. Evidence was also found for the action of the biotic filter, however, convergence patterning in response to the biotic filter was

revealed and contradicts its assumed divergent actions. These results indicated inconsistences in the Response-Effect Framework and ecological strategies, as well as, providing insights into the function responses of temperate grasslands to management strategies.

7.1.3. Chapter Five

A mechanism of understanding and predicting the provisioning of ecosystem processes and services was proposed through the Response-Effect Framework (Lavorel and Garnier, 2002). Despite this, the impact of temperate grasslands' functional structure and composition on goods and services is severely understudied, especially with respect to animal production (Pakeman, 2014b). Two hypotheses have been suggested as bridge between plant traits and ecosystem services; the Biomass Ratio Hypothesis and the Functional Diversity Hypothesis (Grime, 1998; Villéger, Mason and Mouillot, 2008). This chapter investigated the relative and combined effects of environmental factors and effect trait hypotheses on the prediction of biomass production and livestock production. It was revealed environmental factor and both hypotheses collectively explain the greatest amount of variation in quantity of green and brown biomass production. In essence, environmental and management pressures resembling intensely managed temperate grasslands and an exploitative community best supported greater amounts of biomass production. Producing high quality biomass was shown to be underpinned by environmental factors and the functional diversity hypothesis. The results supplement those of biomass quantity and indicate the use of nitrate-based fertilisers to increase the quality of biomass. It was revealed that higher yields from cattle and sheep were accurately predicted from diverged grazing pastures traits and provided support for the Functional Diversity Hypothesis. A trade-off was discovered between producing heavier cattle and high-quality meat. Collectively, the results provided support for Response-Effect Framework and offered novel insights into the provisioning of livestock production from temperate grasslands.

7.1.4. Chapter Six

Understanding community assembly and reassembly has been of paramount importance to predict the trajectory of an ecological restoration project. Functional ecology has provided a medium to model species' relative abundances according to plant trait values via the Maximum Entropy Model, however, the validity of mature plant traits has been questioned (Shipley, 2010). Furthermore, the impact environmental and management decisions have on the structure and composition of seed and germination traits is grossly ill-defined due to a general shortage in measurements (Kattge *et al.*, 2011). This chapter examined the accuracy of mature, seed and germination traits in predicting species relative abundances using the MaxEnt model. A combination of all traits was found to predict species' relative abundances to a high degree (97%) and seven traits was found to be optimal. It was discovered that ruderal communities developed on strong agricultural soil legacies and in response to sowing of ResB; weak competitors and opportunistic weedy plant species were identified as the dominant vegetation type. Later successional species were found to dominate environments where soil nutrient status was diminished, absence of a weedy seed bank and where Loc or ResA were sown. A weedy seed bank and high soil fertility were also indicated as the main effects hindering the progression towards vegetation and functional targets. Together, the results advocated for further study into modelling approaches employing seed and germination traits and focussed efforts on eliminating the agricultural soil legacies will result in longer lasting communities that resemble vegetation and functional targets.

7.2. Addressing Response and Effect Traits

The Response-Effect Framework conceptualized response and effect trait as separate entities (Lavorel and Garnier, 2002). Ecological strategies and published literature, however, appears to treat these separate concepts as synonymous. Plant height, for example, has been linked to ecological strategies of shading and grazing together with scaling up to support biomass production (Franklin, 2008; Schumacher and Roscher, 2009; Dirks *et al.*, 2017). Pakeman (2011) stated the availability of plant trait data limits the investigation of response and effect traits as individual concepts. Furthermore, analyzing the links between response and effect

traits provides a means of estimating the impacts of environmental parameters on ecosystem functions, processes and services simply. A clear causal pathway was identified that links species changes in response to environmental change to ecosystem function, and was suggested to exemplify the Response-Effect Framework (Pakeman, 2011). This thesis found clear evidence in the overlapping of response and effect traits, particularly with leaf traits. However, this thesis suggests the concepts of response and effect are redundant with regards to informing research or keeping the concepts isolated. This thesis postulates revising the Response-Effect Framework, with attention paid to key problems outlined throughout this thesis, such the overlap in response and effect traits. Other issues include considering results where the biotic filter is found to be a divergent rather than a convergent force (Chapter Four) or the functional diversity is found to negatively impact the provisioning of ecosystem processes and services (Chapter Five).

7.3. Databases and Intraspecific Variation

The trait-based approach used in this thesis was, overall, successful in satisfying the overall aim, and sub-aims set out in this thesis. A critical point of contention is the use of average traits values derived from databases and the assumption that interspecific differences are of greater importance than intraspecific differences. Cordlandwehr *et al* (2013) found that database values show a high degree of variation depending on the trait. Further, the differences in database values with onsite measurements of leaf dry matter content was found to be 14mg/g (Duru *et al.*, 2010). Leaf dry matter content is suggested to be mediated by air temperature – a one-degree Celsius increase led to a decrease of 8mg/g. Trait plasticity is eliminated when average trait values are calculated from database recordings.

Measuring trait plasticity and intraspecific variation in experimental settings has been urged by functional ecologists to advance the understanding of ecological axes of specialisation, the impact of climate and environmental factors on trait values, and how these scale to the provision of ecosystem services. The current ecological axes of specialisation have been questioned when considering intraspecific trait correlations (Laughlin and Messier, 2015). Additionally, intraspecific variation

confers niche breadth across environmental gradients (Violle and Jiang, 2009). Intraspecific variation is fundamental to the functional diversity hypothesis and is suggested to play a greater role than interspecific variation in homogenous environments (Albert *et al.*, 2010). Albert *et al* (2010) concluded that the importance of intraspecific variability is dependent on the spatial and organisational scale of the investigation. At macro and global scales, interspecific trait variation is assumed to outweigh intraspecific differences. Furthermore, increasing spatial scale is thought to introduce a greater number of environmental pressures that may lead to false conclusions from intraspecific differences (Siefert, Fridley and Ritchie, 2014). The largest gap in intraspecific trait variation research is their influences on ecosystem processes and services. The functional diversity hypothesis postulates that greater intraspecific trait variation will lead to enhance provision of ecosystem services. Functional ecologist, however, are a few paces behind in trying to identify what plant traits elicit higher degrees of intraspecific variation and through what mechanism (adaptation or acclimation) (Albert *et al.*, 2011). The Leaf Economics Spectrum and its associated traits are suggested to be phenotypically plastic. Specific leaf area was found to discrepancies of 13-30% within a species and leaf dry matter content between 8-20% (Harzé, Mahy and Monty, 2016). Seed mass, on the other hand, is postulated to be highly conserved intraspecifically and questions could be asked of the higher order taxonomies, such as genus, family and order (Lord, Westoby and Leishman, 1995). This has been termed phylogenetic conservatism and has gained wider recognition in recent years.

7.4. Functional Ecology and Phylogenetic Corrections

In recent years, functional ecologists have challenged the use of functional trait diversity in circumstances where sophisticated or appropriate trait measurements are lacking (Flynn *et al.*, 2011; Penone *et al.*, 2014). The calculation of phylogenetic diversity has thus been proposed to resolve trait data inadequacies and as a cost efficient alternative for conservation and restoration managers; the most widely implemented phylogenetic diversity index (Faith's Phylogenetic Diversity) can be calculated solely from presence/absence data (Montoya, Rogers and Memmott, 2012; Mouquet *et al.*, 2012). Additionally, phylogenetic diversity is suggested to summarise a community's ability to respond to environmental changes; high diversity is equal to increased evolutionary potential and resilience (Petchey and

Gaston, 2006). In essence, phylogenetic testing and subsequent corrections are required when there is an increased probability for species to retain an ancestral ecological characteristic based on its phylogeny (Flynn *et al.*, 2011; Schrodt *et al.*, 2015). Pavoine and Bonsall (2011) strongly advocated in using phylogenetic diversity as a complementary index with taxonomic diversity, and the lack of guidelines to help researchers identify under what circumstances phylogenetic diversity should be tested and corrected for (de Bello *et al.*, 2015).

At the simplest level, the relationship between phylogenetic diversity and functional diversity is postulated to unveil phylogenetic signatures in communities (Johnson and Stinchcombe, 2007). A positive relationship indicates a signal of phylogenetic niche conservatism – plant trait(s) is causing closely related species to more similar ecologically than would be expect – suggesting the sole use of phylogenetic diversity (Pavoine and Bonsall, 2011). No relationship, on the other hand, speculates that the plant traits were independent, or there's a mixture of labile and conserved traits, and phylogenetic diversity should be integrated to enrich analyses (Cadotte, Carscadden and Mirotchnick, 2011; de Bello *et al.*, 2015). The need for phylogenetic correction is also thought to depend on the strength of the signal, which is found to be dictated by the spatial scale and community type (Mouquet *et al.*, 2012; de Bello *et al.*, 2015). This ambiguity has resulted in the close examination of individual plant traits and ecological strategies to highlight those believed to be highly conserved.

7.3.1. Individual Traits and Ecological Strategies

Lavorel *et al* (2002) stated that ecological axes of specialisation may be falsely interpreted due to common lineages and since it has been assumed that most traits will exhibit some degree of phylogenetic signal (Pavoine and Bonsall, 2011). Phylogenetic signals have been investigated using the R function *Phylomatic* of the package *picante* but other researchers have inferred phylogenetic inertia from ordination techniques (Reese, Ames and Wright, 2016; Rossatto and Franco, 2017). Rossatto and Franco (2017) concluded that the clear separation of palms from trees and shrubs along a principal component was a clear indication of distinct phylogenetically conserved leaf syndromes. Studies are beginning to emerge to

determine the extent and identity of which trait values are phylogenetically conserved, concentrating on the ecological strategies previously defined; size axis and leaf economics spectrum (Johnson and Stinchcombe, 2007).

7.3.1a. Leaf Economics Spectrum

The core and associated traits of the leaf economics spectrum have been the most widely studied. A number of publications report a lack phylogenetic signals for specific leaf area, leaf dry matter content, leaf thickness, ash content and leaf toughness (Peter J Wilson, Thompson and Hodgson, 1999; Comita and Hubbell, 2009; Reese, Ames and Wright, 2016). However, there is also evidence for phylogenetic conservatism in specific leaf area, size and thickness (Flynn *et al.*, 2011; Kembel and Cahill, 2011). Despite the evidence for phylogenetic conservatism, leaf traits are generally believed to be phylogenetically labile, plastic in nature, and this has manifested in research accounting for intraspecific variability (Rosbakh, Römermann and Poschlod, 2015).

7.3.1b. Size Axis

Plant traits associated with the size axis (plant height and seed mass) have been shown to be phylogenetically conserved (Cadotte *et al.*, 2009; Flynn *et al.*, 2011). In support of this, Bainard *et al* (2012) found that DNA content exhibited a strong phylogenetic signal. Genome size has previously been closely associated with seed mass, and thus suggests phylogenetic conservatism may be consistent with ecological strategies (Grime, Hodgson and Hunt, 2007). The underpinning ecological strategy suggests larger genomes and seeds are better equipped to withstand environmental perturbations and a strong phylogenetic signal has also been reported for endoreplication (polyploidisation) (Westoby *et al.*, 1996; Bainard *et al.*, 2012). Endoreplication has also been indicated to play a modulating role to stress responses (Cookson, Radziejwoski and Granier, 2006).

7.3.1c. Root Traits

The quantification of belowground plant traits is considerably lacking in functional ecology. Valverde-Barrantes *et al* (2017) outlined a root trait phylogenetic conservatism hypothesis and recommended using phylogenetic diversity in difficult circumstances, such as microbiota associations (de Bello *et al.*, 2015). They found that mycorrhizal colonisation (arbuscular mycorrhizal and ectomycorrhizal fungi) displayed a strong phylogenetic signal suggesting that mutualistic interactions are phylogenetically conserved (Valverde-Barrantes *et al.*, 2017). Phylogenetic conservatism in biological interactions (mutualism, parasitism, etc.) has been attributed to co-evolution and evidence has been reported by other researchers (Mouquet *et al.*, 2012). Phylogenetic signals were found for nitrogen fixing bacteria associations and nitrogen fixation (Cadotte *et al.*, 2009; Flynn *et al.*, 2011).

One great benefit resounding from investigations into phylogenetic conservatism has seen improvements in the approaches dealing with missing values in data analyses (Penone *et al.*, 2014). Using a phylogenetic comparative approach, such as hierarchial probabilistic matric factorisation modelling, phylogenetic imputation was found to improve the accuracy for predicting missing traits for plants in the TRY database, whilst maintaining known allometric and trade-off relationships with other traits (Shan *et al.*, 2012). Penone *et al* (2014) reported that use the phylogeny did not decrease the quality of trait imputation and therefore should be used whenever possible. However, the literature reviewed previously and other scholars postulate that phylogenetic imputation is pointless as many traits don't exhibit a phylogenetic signal (Swenson, 2014). Mouquet *et al* (2012) suggested a more productive avenue is to identify traits with strong phylogenetic signals that are constrained by their environmental characteristics; a notion echoed by Swenson (2014). These inferences question the underlying effects of phylogenetic conservatism on the relationships between environmental pressures and response traits.

7.3.2. Response Traits

The convergence-divergence paradox has been key to understanding the functional responses of communities to abiotic and biotic filtering processes. Abiotic filters are

assumed to cause convergence in plant trait values and the biotic filter divergence via the limiting similarity hypothesis that suggests less competitive differentiate to facilitate co-existence (Lavorel and Garnier, 2002; Funk *et al.*, 2008). This convergence-divergence paradox has been applied to phylogenetic conservatism; the synonymous concepts of phylogenetic clustering and overdispersion (Johnson and Stinchcombe, 2007; Hillerislambers *et al.*, 2012). To fully disentangle the influences of phylogeny, researchers have suggested incorporating a phylogenetic diversity index into multi-trait analyses and/or applying a phylogenetic correction in single trait investigations (de Bello *et al.*, 2015). Such approaches have generally revealed weak effects of phylogeny along environmental gradients and concluded that phylogenetic information is less important for trait responses to short term environmental changes (de Bello *et al.*, 2015; Dwyer and Laughlin, 2017; Valverde-Barrantes *et al.*, 2017). Phylogeny was found to not influence a leaf economics spectrum based model of habitat filtering (Welsh, Cronin and Mitchell, 2016). However, the decoupling of phylogenetic contributions and abiotic filtering was found to unmasked local differentiation patterns in mesic meadows from central Europe, which was essential in unveiling the combined actions of abiotic and biotic filtering with communities (de Bello *et al.*, 2017).

Mouquet *et al* (2012) proposed the amalgamation of phylogenetics and community assembly rules into ecophylogenetics. This integrates phylogenetic data to assess the likelihood of community assembly scenarios, predominantly regarding interspecific competition dynamics of co-existence and invasion (de Bello *et al.*, 2017). De Bello *et al* (2013) found that similar assembly patterns were found in multivariate functional and phylogenetic diversity patterning, which suggested that species coexistence strategies may be reflected in phylogenetic diversity. This was reiterated by Carboni *et al* (2016), who reported that phylogenetic similarity was an excellent proxy for interspecific competition driving invasion success.

7.3.3. Effect Traits

The influence of biodiversity on ecosystem services was conceptualised by the Response-Effect framework via effect traits (Lavorel and Garnier, 2002). In many circumstances, ecosystem processes and services are affected by multiple, and

potentially unquantified, traits and thus phylogenetic diversity has been used as a surrogate in these instances (de Bello *et al.*, 2015). Phylogenetic diversity was found to be superior to species richness and functional group richness in explaining plant community productivity (41.5% total variation) (Mouquet *et al.*, 2012). Phylogenetic diversity has also been reported to be an effectively explain variability in community productivity and biomass production (Cadotte *et al.*, 2009; Flynn *et al.*, 2011). Despite these early efforts, more sophisticated measures of functional diversity (FRic, FEve and FDiv) were reported to have similar abilities in predicting biodiversity effects on ecosystem processes and services (Flynn *et al.*, 2011). Mouquet *et al* (2012) questioned which facets of phylogenetic diversity (alpha, beta and gamma) matters the most and how their predictive powers compare to functional diversity.

7.3.4. The Future of Phylogenetic and Functional Diversity

The far-reaching effects of phylogeny has been realised in every facet of functional ecology and therefore substituting functional trait for phylogenetic diversity is very restrictive (Pavoine and Bonsall, 2011; Funk *et al.*, 2016). Researchers are continually recommending to present analyses with and without phylogenetic correction and diversity to unveil conservatism in individual traits, their responses to environmental factors and the scaling of traits to ecosystem processes and services (de Bello *et al.*, 2015, 2017). To merge these analyses, however, a unifying vocabulary, mathematical indices and statistical approaches is required (Pavoine and Bonsall, 2011).

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